DISTRIBUTION AND ECOLOGY OF LARVAL HYDROPSYCHIDAE (TRICHOPTERA) IN NEWFOUNDLAND

CHERIE-LEE FIETSCH

NOTE TO USERS

DISTRIBUTION AND ECOLOGY OF LARVAL HYDROPSYCHIDAE (TRICHOPTERA) IN NEWFOUNDLAND

by

© Cherie-Lee Fietsch

A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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ABSTRACT

Hydropsychidae are represented in Newfoundland by only eight species compared to 145 species in North America. It was predicted that these eight widely distributed hydropsychids would differ in their distribution and ecology here because of the reduced species diversity and the broad diversity of lotic habitats available in the glacial-fluvial morphology of Newfoundland streams. Specifically, that Newfoundland species would differ in their physical niche and that water temperature and food resources would influence their distribution.

Distribution of Newfoundland Hydropsychidae was strongly influenced by lake outlets, which had higher nutrient concentrations and warmer temperatures than downstream sites. All species occurred in forested and barren landscapes. Densities of hydropsychids were elevated at outlets and in forested streams. *Parapsyche apicalis* was restricted to cooler streams. Stream size also influenced the distribution of some species. Other physical factors did not correlate with densities. The hypothesized reduced spatial and nutritional competition between species because of the impoverished fauna did not translate into an expanded habitat range in Newfoundland.

A logistic model was derived to provide a basis of comparison for hydropsychid, plankton and periphyton abundances amongst streams of different sizes. Across stream comparisons showed rapid changes near outlets. Abundances of *C. pettiti* and *H. betteni* declined rapidly below outlets, *H. slossonae* had a fairly constant longitudinal abundance and *H. sparna* increased in abundance downstream. Zooplankton, *H. betteni* and total hydropsychid abundance showed similar longitudinal trends.

All Hydropsychidae species had high proportions of a storage lipid and a fatty acid composition dominated by 14 fatty acids. The fatty acid composition of *P. apicalis* was the most distinctive from the other species, followed by *A. ladogensis, D. modesta* and *H. alternans.* Discrimination of four commonly occurring and most abundant species (*C. pettiti, H. betteni, H. sparna, H. slossonae*) was more difficult indicating the similarity in their fatty acid composition. Changes in lipid and fatty acid composition of seston suggested an influence of lake seston on the lotic community. This study indicated selective feeding differences by hydropsychids, but also demonstrated their ability to adapt to differing food sources among and within streams, showing that hydropsychids are opportunistic generalists.

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LIST OF ABBREVIATIONS & SYMBOLS

AH	Above Hatchet
alt	*Hydropsyche alternans*
AMPL	Acetone Mobile Polar Lipids
arct	*Arctopsyche ladogensis*
BC	Broad Cove
bett	*Hydropsyche betteni*
BK	Barking Kettle
BP	Beaver Pond
BRN	Barren
chm	*Cheumatopsyche pettiti*
dinofl.	Dinoflagellate(s)
dplc	*Diplectrona modesta*
DS	Downstream
FRS	Forested
GC	Goat Cove
GP	Great Pond
HUFA	Highly Unsaturated Fatty Acid
Lev./Leveille	refers to Leveille et al. (1997)
LO	Lake Outlet
MUFA	Monounsaturated Fatty Acid
NB	Nagles Brook
para	*Parapsyche apicalis*
PC	Portugal Cove
PCA	Principle Component Analysis
PL	Phospholipid
PUFA	Polyunsaturated Fatty Acid
SAFA	Saturated Fatty Acid
slss	*Hydropsyche slossonae*
spr	*Hydropsyche sparna*
SR	Split Rock
TAG	Triacylglycerol
WT	Watern

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"The delver into nature's aims seeks freedom and perfection; Let calculation sift his claims with faith and circumspection." Goethe

1. CHAPTER 1: INTRODUCTION TO THESIS

1.1 Overview of Study Objectives

The purpose of the present study was to discern the broad scale distribution of an impoverished fauna in a diverse riverine templet. The profile of Newfoundland stream systems differs from classical systems which have a smooth gradient from headwaters to mouth. Glaciation and resistant lithology have resulted in streams with multiple lentic bodies throughout their length, poorly sorted substrates and slightly acidic oligotrophic water. The glacial-fluvial pattern of Newfoundland's stream systems influences the distribution patterns of benthic macroinvertebrates (Larson & Colbo 1983). For example, the presence of lentic bodies and the size of stream influenced the distribution of blackfly larvae in Newfoundland (McCreadie & Colbo 1992). The fauna of interest here is the Hydropsychidae, represented in Newfoundland by only eight species, as compared to a total of 145 species in North America. It is predicted that hydropsychids (common name for the Family) will be influenced by the physical characteristics of Newfoundland streams.

Hypotheses formulated for this study were:

$\mathbf{H_O}$: The distribution of Newfoundland Hydropsychidae is not different from that of the mainland.

$\mathbf{H_A}$: There are differences in the physical habitat niche of Newfoundland species compared to their reported habitat on the mainland.

$\mathbf{H_O}$: The distribution of Newfoundland Hydropsychidae is not influenced by water temperature and food resources.

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Sub $\mathbf{H_O}$: Hydropsychidae distribution and abundance are not influenced by: 1) lake outlets, 2) terrestrial vegetation patterns, 3) stream size, 4) time, 5) water temperature. Abundances of the potential food resources emanating from lakes and Hydropsychidae abundance do not follow a similar rate of decline away from lake outlets.

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Sub $\mathbf{H_O}$: Lipid class and fatty acid composition, indicative of food resources, of Hydropsychidae do not differ: 1) among species, 2) between larvae and pupae, 3) within species with respect to location, landscape, stream and season, 4) among species within a given site, 5) compared to their food resources in the seston.

Sub $\mathbf{H_A}$: Lipid class and fatty acid composition, indicative of food resources, of Hydropsychidae do differ: 1) among species, 2) between larvae and pupae, 3) within species with respect to location, landscape, stream and season, 4) among species within a given site, 5) compared to their food resources in the seston.

The distribution of Hydropsychidae in Newfoundland is not well known. Few studies have been carried out on the broad-scale distribution of this Family. This study explores the concept of niche breadth, in terms of physical habitat, in a region of abundant freshwater habitat, but with an impoverished hydropsychid fauna. The niche of an organism is its "place in the biotic environment, its relation to food and enemies" or the description of the organisms role within the community (Krebs 2001). The fundamental niche of a species is the maximum range of resources that can be exploited. The realized niche is the extent to which these resources are used, which is generally reduced from the fundamental niche because of competition with other species (Krebs 2001). The reduction in hydropsychid fauna on the Island could translate into a broader realized niche breadth (the range of resources that a species can exploit, here referring to the occupation of a lotic system measured in terms the physical characteristics of the habitat) due to the lack of competition.

Attempts to define the habitat characteristics of a species need to proceed with caution as the broad structure of stream communities is influenced by historical and regional processes (Vinson & Hawkins 1998). A historical concept called the "Ghost of Competition Past" proposes that a niche is maintained through diffuse competition, meaning that they are a consequence of past and present interspecific competition (Connell 1980). Thus a depauperate hydropsychid fauna may not have wider niche breadths because of past competitive interactions. An example of a regional process in Newfoundland is the progressive decrease from 12 species of Plecoptera (stoneflies) on the west coast to only five species on the east coast. Larson & Colbo (1983) partly

attributed this to increased distance from the mainland source of these species as well as relief patterns and chemical characteristics. Such regional differences must be considered when undertaking broad spatial scale studies. Regional differences in the hydropsychid community across Newfoundland have not been documented.

Understanding community composition across landscape scales is a major challenge confronting stream ecologists (Poff 1997). Ecological research on Hydropsychidae, including reports on Newfoundland species, has traditionally been carried out on a single stream system or on a few systems in close geographic proximity (Gordon & Wallace 1975; Hauer & Stanford 1982b; Hildrew & Edington 1979; MacKay 1984; MacKay 1986). Few studies have examined the ecology of species across broad spatial scales and differing landscapes (Kjærandsen 2005; Ross 1963; Smith et al. 2002).

Sampling of numerous stream systems is necessary to examine the occurrence and abundance of the biota in the context of three physical stream characteristics: presence or absence of a lake outlet, stream width and surrounding vegetation patterns. A literature review showed that these factors influence the distribution of hydropsychids on the mainland; for outlet presence/absence and width (Parker & Voshell 1983; Ross & Wallace 1982; Ross & Wallace 1983; Valett & Stanford 1987); for presence/absence of forest cover (Smith et al. 2002).

The physical morphology of Newfoundland streams challenges popular theories of stream ecology. For example, the River Continuum Concept (Vannote et al. 1980) is a holistic, multidisciplinary approach to stream ecosystem theory. It considers how streams function ecologically assuming a continuous gradient from headwaters downstream,

generalizing longitudinal changes in stream physical characteristics and their effects on biological communities. It is an extension of the habitat templet idea of Southwood (1977; 1988) with its main premise being that the longitudinal physical structure of the stream serves as a templet for biological strategies. Newfoundland streams do not exhibit a continuous gradient from headwaters to mouth and so the applicability of this concept is questionable. Newfoundland streams have a high degree of discontinuity and can be viewed as a series of 'patches' as suggested by the concept of Patch Dynamics (Pringle et al. 1988) where a patch is a given unit of a stream, in time and/or space, as determined by the biota and problem in question. Using patches as basic building blocks, these can be fitted into a hierarchical structure, as suggested by Poole (2002), whose model of Hierarchical Patch Dynamics (HPD) views ecosystems as "nested, discontinuous hierarchies of patch mosaics". Patches are nested at spatial and temporal scales and so may be a more appropriate model for Newfoundland streams as it accommodates their high variability.

Review of the literature suggests that hydropsychids partition their niches based on water temperature (Cudney & Wallace 1980; Edington & Hildrew 1973; Hildrew & Edington 1979; Stanford et al. 1988) and food resources (Cudney & Wallace 1980; Fuller & MacKay 1980a; Georgian & Wallace 1981; Hauer & Stanford 1981; Malas & Wallace 1977; Rhame & Stewart 1976) and thus these two factors are hereafter investigated in Newfoundland streams. Hutchinson (1959) suggested that competition for food was the basis for the formation of niche theory; that food relations contribute to the understanding of the intricacies of any ecosystem and are an important aspect of evolutionary ecology.

For these reasons the study of the potential food resources of these eight species is considered in detail in relation to their abundance throughout this study.

If species' distributions are largely governed by accessible food resources, then the occurrence of a species will be dictated by its ability to colonize habitats with an adequate food supply. Lake outlets have increased seston abundance, and thus are able to support a large number of filter feeding insects such as Hydropsychidae (Richardson & MacKay 1991). The riparian vegetation also influences nutrient levels by contributing allochthonous organic matter. Riparian vegetation in Newfoundland drainage basins ranges from forest to shrubs and herbs to open wetlands. Thus there is the opportunity to examine landscape as a factor influencing Hydropsychidae distribution. Very little research has been conducted on the aquatic macroinvertebrates in barren Newfoundland landscapes, and comparisons with forested landscapes are rare.

1.2 Thesis Format

The formatting of this thesis follows that of the journal Hydrobiologia. It has been written in chapter format with each chapter building on the previous ones, starting with this introductory Chapter 1. Study sites and methods are given where first appropriate and then referred to in following chapters.

- Chapter 2 is the macrodistribution of Newfoundland Hydropsychidae in terms of their occurrence and abundance.
- Chapter 3 is the influence of temperature and food quantity, explored at a subset of the previous study sites.

- Chapter 4 is the longitudinal distribution of the fauna and the quantity of their potential food sources from outlets to downstream. A model was derived for sampling this subset of eight rivers so that longitudinal comparisons were possible amongst rivers.
- Chapter 5 considers the lipid composition of hydropsychids to determine diet differences among the species. It explores the effect of the presence of a lake outlet and the effect of vegetative patterns on food uptake.
- Chapter 6 examines the lipid composition of freshwater stream seston, also in terms of the presence of a lake outlet and the effect of vegetative patterns.
- Chapter 7 compares the lipid composition of hydropsychids and freshwater seston.
- Chapter 8 is a summary of the thesis and suggests future research directions.

1.3 Introduction

The following sections review the literature starting with the general role of macroinvertebrates in stream systems and the basic biology of the Hydropsychidae. Factors influencing species segregation are considered as these are important to community composition. Then the fauna of Newfoundland is introduced including a description of known niches on the mainland as compiled from the literature. This is followed by a description of Newfoundland streams, climate and vegetation. These details provide a background and context for the material in subsequent chapters.

1.4 The Role of Macroinvertebrates in River Systems

Over time, streams and rivers have greatly affected the landscape of the earth by their strong erosive capabilities, transport of materials and subsequent alteration of terrestrial habitats. As a source of freshwater, they are fundamental to human existence. Understanding their structure and function has led to the development of stream ecology (Hauer & Lamberti 1996).

Several thousand species comprise the global macroinvertebrate community in river systems, covering a diverse range of phyla. Most are associated with substrates of the stream bottom and are thus referred to as benthos or the macrozoobenthos. Macroinvertebrates are ubiquitous in lotic freshwater ecosystems, being a dominate conduit of energy between primary producers and higher trophic levels such as fish (Giller & Malmqvist 1998).

Insects are a major group of lotic macroinvertebrates and are the focus of this research. There are several orders where all species have at least one obligatory freshwater stage: Odonata (dragonflies and damselflies), Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies) and Megaloptera (alderflies, fishflies, dobsonflies, hellgrammites). Several other orders have at least one species which is aquatic or semi-aquatic during its life cycle: Diptera (e.g. midges, craneflies, blackflies), Coleoptera (beetles), Lepidoptera (butterflies and moths), Hemiptera (true bugs), Hymenoptera (bees, wasps), Collembola (springtails) and Neuroptera (spongillaflies) (Merritt & Cummins 1996). Note that adult stages of most aquatic insects are usually short-lived organisms with wings and so occupy a terrestrial niche (Horne & Goldman 1994). Thus the macroinvertebrate community is extremely diverse and greatly

contributes to the flow of organic matter in lotic habitats via feeding and secondary production (Hauer & Resh 1996).

One method of classifying aquatic macroinvertebrates is by functional feeding group, which is an association between their feeding adaptations and nutritional resource categories. Table 1.1 summarizes these groups. There are four general nutritional resource categories in steam ecosystems: 1) coarse particulate organic matter (CPOM) with particle sizes greater than 1 mm composed mostly of plant parts (leaves, needles, etc), large woody debris, macroalgae and vascular plants; 2) fine particulate organic matter (FPOM) with particle sizes between 1 mm and 0.5 μm represented by detritus and microbiota; 3) periphyton, which is predominately algae (or other material) attached to substrates; and 4) prey, all invertebrates captured by predators. Major food sources can also be categorized by origin, either autochthonous (produced within a river system) or allochthonous (imported from riparian or other terrestrial sources) (Merritt & Cummins 1996).

Table 1.1 Functional feeding group classification for aquatic insects. Modified from. Merritt & Cummins (1996).

Functional group (general category based on feeding mechanism)	Subdivision of functional group		Examples of taxa	General particle size range of food (in micrometers)
	Dominant food	Feeding mechanism		
Shredders	Living vascular hydrophyte plant tissue	Herbivores—chewers and miners of live macrophytes	Trichoptera: Phryganeidae, Leptoceridae	$>10^3$
	Decomposing vascular plant tissue and wood—coarse particulate organic matter (CPOM)	Detritivores—chewers, wood borers, and gougers	Diptera: Tipulidae, Chironomidae	
Collectors	Decomposing fine particular organic matter (FPOM)	Detritivores—filterers or suspension feeders	Trichoptera: Hydropsychidae Diptera: Simuliidae	$<10^3$
		Detritivores—gatherers or deposit (sediment) feeders (includes surface film feeders)	Ephemeroptera: Ephemeridae Diptera: Chironomidae	
Scrapers	Periphyton—attached algae and associated material	Herbivores—grazing scrapers of mineral and organic surfaces	Trichoptera: Glossosomatidae Coleoptera: Psephenidae Ephemeroptera: Heptageniidae	$<10^3$
Predators (Engulfers)	Living animal tissue	Carnivores—attack prey, pierce tissues and cells, and suck fluids	Hemiptera: Belostomatidae	$>10^3$
	Living animal tissue	Carnivores—ingest whole animals (or parts)	Odonata, Plecoptera: Perlidae	$>10^3$

1.5 Review of Organisms of this Study

More than 9600 species of Trichoptera are found world wide, belonging to 45 families and 626 genera (Giller & Malmqvist 1998). In North America there are 1340 species, with 145 species belonging to the family Hydropsychidae (Giller & Malmqvist 1998; Wiggins 1996). Trichoptera (caddisflies) literally translates to mean 'hairy-wing', referring to the fine, hair-covered wings that fold over the adult body while at rest. Trichoptera are holometabolous, undergoing complete metamorphosis from larvae into adults and spend most of their life cycle in the larval stage. Adults are typically present near aquatic systems for a few weeks during the spring/summer. Adults mate, females oviposit (often on substrates under the water surface) and die. Eggs develop and hatch into first instar larvae, typically molting through five instars. The mature larvae pupate,

and in the spring/summer emerge as adults. Caddisflies typically have annual life cycles in temperate climates, although some may have more than one generation per year (MacKay 1979; Wiggins 1996).

Trichoptera are a diverse group of freshwater insects. They inhabit a wide range of environments from lakes and marshes to rivers and cold springs. They are fundamental members of aquatic ecosystems, occupying numerous niches and contribute to the processing of organic matter and the nutrient flow in aquatic food webs. They are represented in all the functional feeding groups (grazers, shredders, collectors, and predators). Abundances, and subsequently biomass, of caddisflies can be high, creating an important conduit of energy to larger organisms such as fish, birds and bats. (Heliovaara & Vaisanen 1993; Wiggins 1996).

Trichoptera are one of the major groups of freshwater insects and are often the most species rich and ecologically diverse component of the aquatic insect community with the exception of the Diptera. This great diversity is attributed to the use of silk by larvae. Generally, trichopteran larvae construct a shelter, using silk produced by larval silk glands to weave a wide variety of cases, retreats and feeding structures. Pebbles or woody debris are often incorporated to fortify the structure, and a silk web is spun by filter-feeders to sieve food particles out of the water. The specificity of these shelters allows caddisflies to occupy a number of niches and finely partition aquatic resources. The Trichoptera comprise three suborders: Spicipalpia (closed-cocoon makers), Integripalpia (portable-case makers) and Annulipalpia (fixed-retreat makers). The last is

of interest to this study and consists of seven families (Wiggins 1996), with the family Hydropsychidae being the focus of this research.

The Hydropsychidae are ubiquitous in fresh running waters throughout the world, with more than one genus often represented in a single stream system. North America is home to 10 genera and 145 species of hydropsychids. The larvae of these display characteristic longitudinal distributions in running waters because of their differing preferences for water velocity, oxygen level and temperature (Wiggins & MacKay 1978). They are of ecological importance because of their abundance, high biomass and sensitivity to environmental factors such as temperature, pH, water velocity, sedimentation and oxygenation (Wiggins 1996). Their high level of sensitivity makes hydropsychids indicative of environmental perturbations, which can be monitored to determine the health and functioning of aquatic ecosystems (Cao et al. 1996).

Hydropsychidae exhibit a wide range of pollution tolerance, but when this is exceeded detrimental effects are exhibited. These include irregular mesh sizes of larval nets, blackening of the anal papilla hairs of the larvae and fluctuating asymmetry of larval and pupal morphometric characteristics (Bonada & Williams 2002; Tessier et al. 2000; Vuori 1994; Vuori & Kukkonen 2002).

Hydropsychidae larvae are fixed retreat builders. They build retreats attached to stable substrates within a river using silk and plant or mineral material. At the front of the retreat a frame is made within which they spin a highly symmetrical mesh net (Figure 1.1). The net is orientated into the water flow so that it sieves particulate matter from the water. The larvae clear the net using their mandibles, and ingest trapped material. This

food includes plant and animal material, algae, fungi, and bacteria, some of which may be adhering to other organic materials. The type of material collected largely depends on the mesh size of the net (Ross & Wallace 1983; Wallace & Merritt 1980).

The mesh size of the net is determined by larval morphology and changes with the increasing size of the larvae as they proceed through five instars. Differing larval morphology also means that the same instar of each species constructs nets of differing mesh size. Changes in mesh size result in partitioning of resources, possibly contributing to the ability of several species to inhabit a given reach of a river (Alstad 1980; Fey & Schuhmacher 1978; Tachet et al. 1987).

Larvae can be territorial, keeping nets at least a larval body length apart, because larvae may reach that far out of their retreats to attack neighbouring larvae. Usually the smaller larva or the one trying to establish its retreat relinquishes. Territoriality seems to be dependent on competition for space and food resources (Matczak & MacKay 1990; Wiggins 1996). Larvae are capable of producing sound via stridulation by rubbing a dorsal projection on the profemur against a ridged area on the ventral side of the head. The behavioural implications of this are speculative; Jansson & Vuoristo (1979) suggest stridulation is used to defend retreats from intruders, with a larger number of sound bursts correlated with larger intruder body size. Johnstone (1964) suggested two possible functions of stridulation, either as a defensive action against predators and intruders or as a territorial behaviour that affects larval density of hydropsychids (Jansson & Vuoristo 1979).

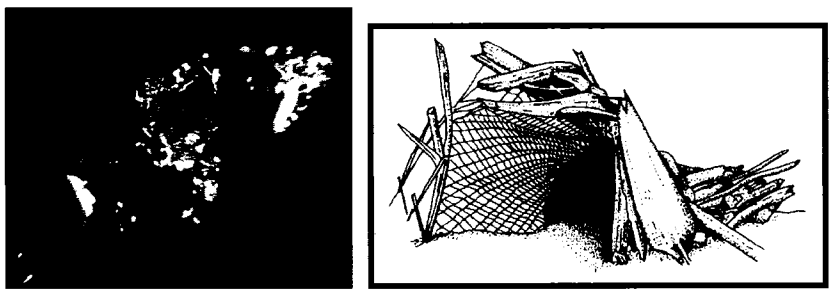

Figure 1.1 Retreat and net of Hydropsychidae. From Wiggins (1996) & www.benthos.org

Warm-water species of Hydropsychidae may be multivoltine, completing up to three generations per year (MacKay 1979; MacKay 1986; Rutherford & MacKay 1986). Species in more northern regions with cooler water temperatures generally complete one generation per year, with the larval stage occupying much of that time (Solem & Gullerfors 1996). The first instar larvae of univoltine populations hatch in the summer, July/August, and reach the third or fourth instar by late fall when water temperatures decline (MacKay 1984). Over winter the larvae are thought not to feed, remaining in a dormant state with metabolic processes greatly slowed by low temperatures (MacKay 1979; Rhame & Stewart 1976). As the water warms in the spring larvae start to feed and reach the fifth instar by late spring or early summer. The larvae then build a cocoon by barring off the ends of the retreats, leaving holes for water flow (Wiggins 1996). In low oxygen conditions the larvae undulate their bodies within their retreats to increase water flow over the ventral gills (Wiggins 1996). They pupate, shedding their entire cuticle and gut lining. Pupae also aid oxygen uptake by undulation of their bodies to increase water flow over their gills, reduced in surface area from those of the larvae (Rhame & Stewart 1976). After a few weeks the pupae complete metamorphosis and cut themselves out of

their cocoons using their pupal mandibles. They move to the surface of the water, and molt to an adult. The adults then mate and the female lays her eggs on a substrate under the surface of the water. The eggs quickly develop and hatch into first instar larvae (Wiggins 1996). Adults generally live three to 15 days and their life spans can be increased experimentally by feeding them sugar-water (Fremling 1960).

Longer adult life spans increase the possibility of wind dispersal. Trichoptera have been recorded "many miles out at sea" (Johnson 1969). Wolf et al. (1986) found a hydropsychid adult 74 km off the Texas-Louisiana shore. Flight capabilities of adults are generally unknown (Nimmo 2003), although Solem & Gullerfors (1996) stated that Hydropsychidae are strong fliers because of their swarming behaviour. Kovats et al. (1996) found inland dispersal of adults to be limited with distances traveled by *Cheumatopsyche* and *Hydropsyche* averaging approximately 660 m and 1590 m respectively. This was determined using a series of light traps. In contrast, Milne (1943) suggested that adults have poor flight abilities based on their restriction to local areas.

Hydropsychid larvae can have very high rates of production compared to other stream benthic macroinvertebrates (Huryn & Wallace 2000). Exceptionally high biomasses have been measured at lake outlets which has been attributed to warmer temperatures and nutrient rich plankton emanating from the lake (Huryn & Wallace 2000). High hydropsychid biomass has also been measured throughout streams in the Applachian Mountains, where *Parapsyche* had high biomass measures in upstream reaches and *Cheumatopsyche* and *Hydropsyche* had high biomass measures in downstream reaches (Grubaugh et al. 1996; Grubaugh et al. 1997).

1.6 Review of Species Segregation

Multiple species of Hydropsychidae co-exist in streams throughout the world. There are several factors thought to explain such co-existence, and one or more of these factors may act simultaneously. Factors include temperature, water velocity, microhabitat selection, proximity to a lake-outlet, food quantity and quality, and life history and growth rates. Species are thought to partition their resources by differences in mesh size and temporal sequencing of life stages (Cudney & Wallace 1980; Fuller & MacKay 1980b; Georgian & Wallace 1981; Hauer & Stanford 1981; Malas & Wallace 1977; Rhame & Stewart 1976).

1.6.1 Temperature

Temperature affects the distribution of Hydropsychidae species both among streams and longitudinally within a stream system, and also influences their growth rates and life cycles (Cudney & Wallace 1980; Hildrew & Edington 1979). Caddisflies are poikilotherms so the environmental temperature affects their metabolic rate and is therefore linked to feeding, growth rates and fundamental physiological processes.

Hildrew & Edington (1979) found temperature to be a major factor influencing the longitudinal distribution of Hydropsychidae in the River Usk, southern Wales. The longitudinal succession of Hydropsychidae species corresponded to changes in the thermal regime from headwaters downstream. In the headwaters of the 125 km long river was *Diplectrona felix*, belonging to a genus which is adapted to cooler temperatures and smaller rivers (Wiggins 1996). Four species of *Hydropsyche* were found downstream, spatially segregated by their temperature tolerance. *Diplectrona* may have a higher

metabolic rate than *Hydropsyche* at warmer temperatures and therefore may not be able to survive at warmer downstream sites (Edington & Hildrew 1973).

The presence of lentic bodies along a river also influences the temperature regime. Lake outflows can be warmer because the lentic body absorbs solar radiation and the less dense warmed water remains at the surface and flows out at the lake outlet. This is influenced by the mixing regime of the lake. Thermal influence of lentic bodies is especially evident below man-made dams which tend to regulate the temperature downstream. If water is released from the top of the dam this can cause increased temperatures downstream, whereas releases near the bottom of the dam can be cooler due to thermal stratification of the dammed water body, but this is influenced by the mixing cycle of the water body (Stanford et al. 1988). In a classical river system, fed only by surface runoff and groundwater, the thermal regime is generally a continuous gradient from mountain headwaters to lowland valleys. The temperature ranges from cool, spring-fed headwaters to much warmer temperatures downstream due to increased air and soil temperature, presence of pools, increased surface area and greater input from surface runoff. The presence of lentic bodies along rivers interrupts this continuous gradient and affects the distribution of hydropsychids (Hauer & Stanford 1982a).

Temperature is also inversely related to dissolved oxygen levels. In addition, increased temperatures raise the biological oxygen demand by increasing respiratory demands of organisms so the available oxygen declines even more (Giller & Malmqvist 1998). Hydropsychids are sensitive to oxygen levels and so the combination of

temperature and dissolved oxygen content partly determine the distribution of these organisms (Philipson 1969).

1.6.2 Water velocity

Osborne & Hendricks (1987) hypothesized that micro-scale flow patterns may be important in influencing the distribution of hydropsychids. Flow dynamics near the surface of hydropsychid nets attached to and among the substrate are very variable and difficult to measure accurately at the organisms scale. A laboratory experiment by Edington (1968) showed *Hydropsyche instabilis* increased the number of nets produced per day at faster water velocities (25 cm/s). However, faster water velocity also increases drag on hydropsychid nets and exerts increased structural stress (Brown et al. 2005). Becker (1987) found *Hydropsyche pellucidula* mesh size to be smaller at lower current velocities. Water velocity is also linked to oxygenation, with faster waters having higher oxygen concentrations (Hynes 1970a).

1.6.3 Proximity to a Lake Outlet

Hydropsychids often occur in high densities at lake outlets, as do other filter feeding macroinvertebrates. Richardson & MacKay (1991) gave a mini-review of lake outlet communities and stated that lake outlet "organisms are responding to a gradient in environmental factors". Lake outlets are potentially a rich habitat, with an abundance of suspended material (seston) from the lake as a potential food source. Hydropsychid densities are often greater at lake outlets than downstream because of this abundance of potential food (MacKay & Waters 1986; Parker & Voshell 1983; Petersen 1987c; Spence & Hynes 1971). Figure 1.2 shows the theoretical change in the density of filter feeding

organisms with increasing distance from lake outlets. At lake outlets, water temperatures are raised, as previously noted. Discharge from a lake outlet is hydrologically more stable, often occurring even when areas downstream may be experiencing no surface flow. Even during periods of heavy rain, outlet discharge does not produce substrate erosion due to the gradual gradient from the lake. Flow is typically laminar, an aspect thought to increase capture efficiency by hydropsychid nets and to prevent damage to fragile plankton, leaving intact cells to be consumed by the larvae (Maciolek & Tunzi 1968). Lake outlets are often wide and shallow (compared to downstream), exposing a greater proportion of the water column to the nets of filter feeders (Morin & Peters 1988). Stable substrates and low sediment content at lake outlets create a favourable habitat for sedentary filter feeders. Reduced scour of the stream bottom allows mats of algae or other aquatic plants to grow, providing additional attachment sites for filter feeders, although they may also reduce suitability by altering flow and substrate type. Thus physical factors contributing to the high density of filter feeders include seston, temperature, flow, depth and substrate.

Biological factors such as competition, predation and colonization cycle will affect community interactions (Richardson & MacKay 1991). Competition is thought to be a driving force in the distribution of hydropsychids (Hart 1983) with high densities at lake outlets sustained by high concentrations of seston (Petersen 1987c). This means that even suboptimal sites, in terms of velocity and depth, have adequate food supply, permitting the co-existence of a higher density of individuals where even close neighbours are tolerated. The effect of predation is low relative to the high survival rate

and recruitment of hydropsychids at lake outlets (Richardson & MacKay 1991). Recruitment is high because gravid females tend to fly upstream to oviposit and so when stream conditions end at the lake outlet the females must lay their eggs (Roos 1957).

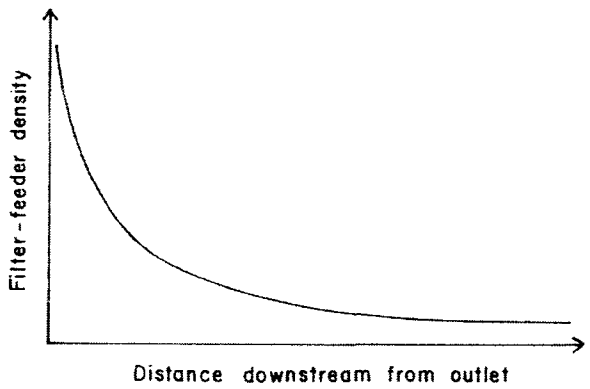

Figure 1.2 A diagrammatic representation of filter feeder density and distance from a lake outlet. From Richardson & MacKay (1991).

1.6.4 Food Quantity and Food Quality

Petersen (1987a) stated that filter feeding caddisflies are common at outlets because they are selective feeders which prefer high quality foods like zooplankton. Lake outlets provide a large quantity of potential food sources as well as a high quality of food. Naiman (1983) defined food quality as the " growth-producing nutritive content per unit mass, whereas food quantity is the density per unit of environment". As seston is carried downstream, its quantity decreases because it settles out of the water column and is consumed by organisms. Nutrients are cycled through organisms and egested material contributes to nutrient sources downstream, a process called nutrient spiraling (Elwood et al. 1983). The quality of food may also decrease with distance from the lake outlet because filter feeders are thought to selectively remove growth promoting particles, a

theory known as the food depletion hypothesis (Giller & Malmqvist 1998; Richardson & MacKay 1991).

The range of particle sizes also differs from lake outlets to downstream and filter feeders are able to exploit these changes (Voshell & Parker 1985). Those at lake outlets may preferentially remove large zooplankton and/or fine particles, thus changing the size spectrum of particles available further downstream. As a river flows along its basin it receives input of particulate organic and inorganic matter such as suspended mineral particles. These become part of the seston but the inorganic particles are not a food source for filter feeders. The quality and quantity of food becomes more diluted with increasing distance downstream where the input of nutritious material is often much lower than that of inorganics. Thus as the dilution hypothesis predicts the quality and/or quantity of food per unit of filtrate is reduced with increasing distance downstream (Richardson & MacKay 1991). Additionally, this increased proportion of inorganic material may clog feeding nets, further hampering food intake. The food depletion and food dilution hypotheses are depicted in Figure 1.3. The food depletion hypothesis (A) predicts that the main effect is the loss of high quality particles with downstream distance, decreasing the concentration of high quality food. In the food dilution hypothesis (B) the greater input of low quality food with distance downstream is predicted to reduce the value of the seston as a food source.

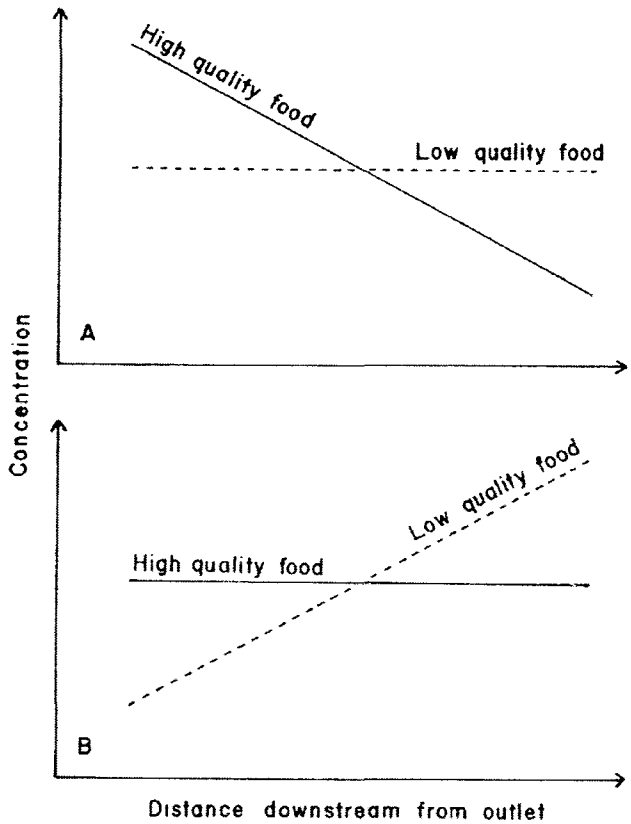

Figure 1.3 The food depletion hypothesis (A) and the food dilution hypothesis (B). From Richardson & MacKay (1983).

1.7 Hydropsychidae of Newfoundland

Only nine species of Hydropsychidae have been documented from Newfoundland as set out in Figure 1.4 (Schefter & Wiggins 1986; Wiggins 1996). These are representative of three of the four subfamilies of Hydropsychidae, the fourth being Macronematinae (Marshall & Larson 1982; Schuster & Etinier 1978).

Suborder: Annulipalpia (fixed-retreat makers)
Family: Hydropsychidae
Subfamily: Arctopsychinae
Arctopsyche ladogensis
Parapsyche apicalis
Subfamily: Diplectroninae
Diplectrona modesta
Subfamily: Hydropsychinae
Cheumatopsyche pettiti
Hydropsyche alternans
Hydropsyche betteni
Hydropsyche slossonae
Hydropsyche sparna
Hydropsyche ventura

Figure 1.4 Newfoundland Hydropsychidae

Newfoundland Trichoptera have been investigated by Marshall (1975), Marshall & Larson (1982) and Genge (1985). Marshall (1975) cataloged the adult Trichoptera of the Island and recorded 97 species, including five Hydropsychidae (*Arctopsyche ladogensis, Hydropsyche betteni, H. slossonae, H. sparna* and *H. alternans*). Genge (1985) studied the distribution and life history patterns of the Trichopteran community in a small river near the city of St. John's. He found *Cheumatopsyche pettiti* and *Hydropsyche betteni* to predominate at the lake outlet, with *H. slossonae* and *H. sparna* predominating 60m downstream from the lake outlet. Life cycles were generally univoltine. He also found a reduction in large seston particles downstream. He concluded that differences in habitat preferences, feeding habits and timing of life stages permitted the co-existence of a guild of net-spinning caddis flies at Axes Pond lake outlet.

1.8 North American Distribution of Newfoundland Hydropsychidae

The Hydropsychidae found in Newfoundland have a widespread distribution across northern and eastern North America (Table 1.2). The eastern range limit in all cases is Newfoundland, which is also the northern range limit for two species, *Hydropsyche ventura* and *Diplectrona modesta*. Maps of the adult distribution in North America for each of the nine species can be found in Appendix 1 (section 10.1); they were created by Nimmo (1987) based on the published literature of collection sites. A literature review determined which stream characteristics most influence hydropsychid distribution in North America. These include stream size, water temperature and water velocity. Table 1.3 gives a brief synopsis of stream characteristics and includes final instar larval length, mesh size and diet with data gathered from multiple sources. Detailed information of the known niche characteristics of each species is outlined. Table 1.2 & Table 1.3 summarize the known distribution and general niche characteristics of Newfoundland Hydropsychidae in North America. These tables are derived from studies based mainly on single river systems.

Table 1.2 Summary of the known North American geographic distribution of Newfoundland Hydropsychidae (Nimmo 1987). Maps are in Appendix 1 (section 10.1).

Species	Map Figure #	Distribution (eastern limit is Newfoundland)			
		Overall	North	South	West
Arctopsyche ladogensis (Kolenati 1859)	10.1	Transcontinental	tree line	Michigan	Alaska
Parapsyche apicalis (Banks, 1908)	10.2	eastern North America	northern Quebec	Tennessee	Wisconsin
Diplectrona modesta Banks, 1908	10.3	eastern North America	Newfoundland	Florida	Oklahoma
Cheumatopsyche pettiti (Banks, 1908)	10.4	Transcontinental	tree line	Texas	British Columbia
Hydropsyche alternans (Walker, 1852)	10.5	Transcontinental	tree line	Wisconsin	British Columbia
Hydropsyche betteni Ross, 1938	10.6	eastern North America	tree line	Georgia	Saskatchewan
Hydropsyche slossonae Banks, 1905	10.7	Transcontinental	tree line	Arkansas	British Columbia
Hydropsyche sparna Ross, 1938	10.8	eastern North America	tree line	Alabama	Manitoba
Hydropsyche ventura Ross, 1941	10.9	Appalachian Mountains	Newfoundland	Tennessee	Tennessee

Table 1.3 Summary of the general niche characteristics of Newfoundland Hydropsychidae. Data are compiled from multiple sources given in the text. Adult emergence times are from Nimmo (1987).

Species	River Characteristics			Final instar length (mm)	Final instar mesh size (µm)	Larval Diet	Adult Emergence
	Size (m)	Temperature (°C)	Velocity (m/s)				
Arctopsyche ladogensis	5 - 15	<18	fast	20+	403 x 534	carnivorous	May to August
Parapsyche apicalis	0.5 - 27	generally < 10, max. of 15-28	< 0.15 to > 0.75	18 - 20	272 x 341	detrital carnivorous diatoms	May to October
Diplectrona modesta	0.5 - 8	1.7 - 24.5, min. growth of 6.5	0.15 - 0.45	15	188 x 243	detrital carnivorous diatoms	May to September
Cheumatopsyche pettiti	small (or larger)	warmer, <30	any	-	77 x 111	algae carnivorous	May to September
Hydropsyche alternans	3 - 75	cool to warm, <28	0.45 to >0.75	-	-	carnivorous	May to September
Hydropsyche betteni	2 - 76	warm preferred, 2.8 to 25.5	<0.15 to 0.75	16	148 x 250	algae diatoms carnivorous	April to September
Hydropsyche slossonae	2 - 25	cool, max 15 - 25	0.15 to >0.75	16	176 x 298	detrital carnivorous diatoms	May to August
Hydropsyche sparna	0.5 - 21	1.7 - 26.5	0.15 - 0.75	-	190 x 300	algae carnivorous diatoms	April to September
Hydropsyche ventura	small to large	cool	fast	-	-	-	April to September

1.8.1 *Arctopsyche ladogensis*

Arctopsyche ladogensis is transcontinental occurring in North America west to Alaska, south to Michigan and north to the treeline (Table 1.2) (Nimmo 1987). This species also occurs in north Europe (Brittain & Bildeng 1995; Englund et al. 1997). Fifth instar larvae are the largest of the Newfoundland species and can be 20 mm or more in length (Flint 1961). This species generally inhabits large (25 m wide and up to 1 m deep), clear, cold, rapid streams with substrates of gravel and boulders (Brittain & Bildeng 1995; Englund et al. 1997; Flint 1961). Mature larvae build a large-mesh net but mesh

size is not reported in the literature. It is thought to be similar to that of *A. irrorata* which has a large mesh size of 403 x 534 μm (Wallace 1975b). In Maine, mature larvae overwintered and pupated in May-June with adult records in June (Flint 1961). In a Norwegian river, Brittain & Bildeng (1995) found adult females to be significantly larger than adult males where two thirds of the population was semivoltine and the remaining univoltine. They found temperature to clearly influence life history with colder temperatures delaying molting. Larvae are primarily carnivorous, feeding on other aquatic insects (Mecom 1972; Wallace 1975b).

1.8.2 *Parapsyche apicalis*

Parapsyche apicalis exists in eastern North America (Flint 1961) occurring north through Quebec, south to Tennessee and west to Wisconsin (Table 1.2) (Nimmo 1987). Larvae are smaller than *Arctopsyche* with fifth instar larvae reaching 18-20 mm in length (Flint 1961). Larvae are generally found in small, cold (<10°C), spring-fed streams but may also be found in large, cold rivers and so temperature has been reported as playing an important role in their distribution (Wiggins 1996). In Massachusetts larvae were multivoltine without defined cohorts where larvae overwintered as many instars, pupae and adults were found throughout the summer and adults occurred from May to October (Flint 1961). It had a similar habitat in Wisconsin (temperature maximums of 15-28°C, widths of 0.5-27 m, currents of <0.15 to >0.75 m/s, sand to cobble substrates, intolerant of pollution, emerging April to August) (Schmude & Hilsenhoff 1986). Pupal cases are almost exclusively constructed of organic matter (Flint 1961), which has also been found for larval retreat construction in Newfoundland (personal observation). Masteller & Flint

(1980b) reported six adults emerging in June-July from a small Pennsylvanian stream in an equal sex ratio. Mesh size is not reported for *P. apicalis* but is thought to be similar to that of *P. cardis,* which has a mesh size of 272 x 341 μm (Wallace 1975b). Larvae mainly feed on fine detritus and animal material (Ross & Wallace 1983). Its diet in Wisconsin streams consisted of animal material and diatoms (79, 21 % respectively) (Shapas & Hilsenhoff 1976).

1.8.3 *Diplectrona modesta*

Diplectrona modesta exists in eastern North America, occurring from Florida in the south to Oklahoma in the west to Newfoundland in the east (Table 1.2) (Marshall & Larson 1982; Nimmo 1987). Mature larvae at 15 mm long are smaller than *Parapsyche* (Ross 1944). They inhabit small, cool, fast flowing streams (Wiggins 1996). It was only found in smaller streams in the Savannah River Basin of the Carolinas and Georgia (Gordon & Wallace 1975) which extended from high altitudes to coastal plains; however in the upper portions it inhabited small to large streams and in the lower reaches it was restricted to smaller streams (temperature 1.7-24.5°C, dissolved oxygen 4.4-12.4ppm). Larvae are sensitive to temperature changes, having a critical minimum growth temperature of 6.5°C (Markarian 1980). Larvae are reported to colonize the bottom of stream substrates (Malas & Wallace 1977). Gurtz & Wallace (1984) found greater abundances in cobble substrates than in sand. In North Carolina it has a univoltine life cycle with high densities (annual mean>200 larvae per m^2) and pupation occurring from May to August (Benke & Wallace 1980; Haefner & Wallace 1981). It had a semivoltine life cycle in Wisconsin, inhabiting small, unpolluted streams (temperature maximums of

16-20°C, widths of 0.5-8 m, currents of 0.15 to 0.45 m/s, sand to cobble substrates, low pollution tolerance, emerging May to July) (Schmude & Hilsenhoff 1986). This was also the emergence period for adults in Pennsylvania (Masteller & Flint 1980b) where the ratio of males to females was alike, although there was a predominance of females in one of the three sampling years. Emergence occurred in late August in Pennsylvania where the sex ratio was alike (Masteller & Flint 1980a). In Illinois emergence occurred from May to June (Ross 1944). Net dimensions were 188 x 243 μm (Wallace 1975b). This species feeds primarily on fine detritus and animal material (Malas & Wallace 1977; Ross & Wallace 1983). Other reports of feeding habits are quite varied: plankton and sessile diatoms (Ross 1944); bacteria (Hynes 1970a); fine detritus (Minshall 1967); detritus, grazing and carnivory (Woodall & Wallace 1972); detritus, animal and diatoms (52:44:4 %) (Shapas & Hilsenhoff 1976).

1.8.4 *Cheumatopsyche pettiti*

The genus *Cheumatopsyche* tends to be more abundant in warmer streams and more tolerant of pollution than the genus *Hydropsyche* (Nimmo 1987). This species has a transcontinental distribution, occurring from Texas to British Columbia to the treeline in the north (Table 1.2) (Nimmo 1987). It inhabits small brooks to large rivers (Ross 1944). Gordon & Wallace (1975) found it in watersheds ranging in size from 13-19446 km^2 (water temperature 3.6-26.5°C, dissolved oxygen content of water 4.8-12.5 ppm). It tolerates a wide range of water temperatures, up to 30°C (MacKay 1986). This is demonstrated in Hawaii where it was inadvertently introduced in the 1960s and is found in riffle habitats in warm streams with moderate to high loads of organic matter

(Kondratieff et al. 1997). This species had a univoltine life cycle in a spring-fed stream in Minnesota where larvae overwintered as instars three and four and pupated in May with eggs hatching in June (MacKay 1986). Abundances are often elevated below impoundments (MacKay & Waters 1986). A population in a small stream in Virginia had a similar life cycle but began pupation earlier as it was bivoltine at upstream reaches and semivoltine at downstream reaches where it had higher abundances (Sanchez & Hendricks 1997). This species is able to occupy the interstitial spaces of gravel stream beds and has been found 20cm deep (Williams & Hynes 1974). Mesh dimensions of this species (formerly named *C. analis*) measured 77 x 111 μm, the finest recorded mesh size of any Newfoundland hydropsychid (Wallace 1975b). Analysis of the gut content of *Cheumatopsyche* showed that it fed on algae (50%) and small animals (40%) with very little detritus (10%) (Coffman et al. 1971).

1.8.5 *Hydropsyche alternans*

Hydropsyche alternans (formerly called *H. recurvata*) has a transcontinental distribution occurring from Wisconsin to British Columbia to the treeline in the north (Table 1.2) (Nimmo 1987). It occurs in a range of habitats from large, fast rivers to lake outlets, and is the only Newfoundland hydropsychid species known to inhabit the wave-washed shores of lakes (Schefter & Wiggins 1986). Ross (1944) reported 14 mm long mature larvae inhabiting swift cold rivers, with adults emerging from May to September in Illinois. It had a univoltine life cycle in Wisconsin, inhabiting a wide range of habitats (temperature maximums of 26-28°C, widths of 3-75 m, currents of 0.45 to >0.75 m/s, cobble/boulder substrates, moderate pollution tolerance, emerging May to August)

including lakeshores (Schmude & Hilsenhoff 1986). In a Saskatchewan lake this species occurred along the shoreline on the underside of rocks where they pupated in July, although all life stages were found in August suggesting a multivoltine life cycle and larvae were reported to be carnivorous (Milne 1943). Mesh-sizes were not reported in the literature.

1.8.6 *Hydropsyche betteni*

Hydropsyche betteni has an eastern North American distribution occurring from Georgia to Saskatchewan to the treeline in the north (Table 1.2) (Nimmo 1987). Ross (1944) reported 16 mm long mature larvae inhabiting riffles of small to medium sized streams as well as shallow swift rills in spillways. It is common in small, warm streams where it can be abundant and is often the most numerous caddisfly present; emergence times were from April to September (Schuster & Etinier 1978). Gordon & Wallace (1975) found this species in a wide range of watershed areas (0.3-414 km^2, temperature 2.8-25.5°C, dissolved oxygen 5.5-11.9 ppm) where it was infrequent in the largest rivers and most abundant on very solid substrates. It occurred throughout Wisconsin except for being absent in very large rivers; it occurred in cold streams but was more abundant in warm streams and in those with "significant organic enrichment" (temperature maximums of 19-30°C, widths of 2-76 m, currents of <0.15 to 0.75 m/s, silt to boulder substrates, high pollution tolerance, emerging May to August) (Schmude & Hilsenhoff 1986). Low numbers of adults emerged (18 specimens) from June to July in Pennsylvania with an equal sex ratio (Masteller & Flint 1980b). This species was bivoltine in a warm southern Ontario river (MacKay 1979). Mesh-sizes for fifth instars were 148 x 250 μm

(Fuller & MacKay 1980a), with an increase in mesh-size for progressive instars. In Wisconsin streams its diet was diatoms, detritus and animal material (66, 20, 14 % respectively) (Shapas & Hilsenhoff 1976). Coffman et al. (1971) reported ~95% of its gut contents to consist of animal material, the remainder consisting of algae in a Pennsylvania woodland stream. In the laboratory, Fuller et al. (1988) fed *H. betteni* leaf detritus, cyanobacteria, *E. coli* bacteria, algae and *Daphnia magna.* Larvae lost weight on all diets except for two types of algae and *D. magna* with the greatest weight gain on the animal material, indicative of the difference in the energy assimilated from these food sources. A second experiment considered temperature, with no larval growth occurring at 5°C, weight gain on all foods at 14°C, and weight gain only on a diet of diatoms and *D. magna* at 20°C which may be indicative of higher metabolic requirements at elevated temperatures (Fuller & Fry 1991).

1.8.7 *Hydropsyche slossonae*

Hydropsyche slossonae has an eastern North American distribution from Arkansas to British Columbia to the treeline (Table 1.2) (Nimmo 1987). It is widely distributed in cool streams (Schefter & Wiggins 1986; Schuster & Etinier 1978). Variations in head colouration occur, with greater deviation in northern ranges (Schefter & Wiggins 1986). Mature larvae are 16 mm long and adults emerge from May to August in Illinois (Ross 1944). In Pennsylvania/Ohio adults emerged in August (Masteller & Flint 1979). In Wisconsin it inhabited a wide range of streams (temperature maximums of 15-25°C, widths of 2-25 m, currents of 0.15 to >0.75 m/s, sand to boulder substrates, moderate pollution tolerance, emerging May to August) but was most common in cold,

small, shallow (8-45 cm), clean streams (Schmude & Hilsenhoff 1986). This species had a univoltine life cycle in a spring-fed stream in Minnesota where larvae overwintered as third instars and pupated in June with eggs hatching in July (MacKay 1986). It overwintered as instars three and four in Virginia, with pupation and emergence occurring from May to June. Mortality was high in the first instar due to sibling cannibalism and was high at the pupal stage possibly due to parasitism by chironomids (Willis & Hendricks 1992). Larvae of this species are preyed upon by stoneflies (Duvall & Williams 2000). It had a bivoltine life cycle in Ontario below an impoundment which was a source of plankton (Fuller & MacKay 1980a; MacKay 1979) and abundances were also greatly increased below impoundments (MacKay & Waters 1986). Abundances of *H. slossonae* in Michigan were positively correlated with algal seston (Fairchild & Holomuzki 2002). In Wisconsin streams its diet consisted of detritus, diatoms and animal material (57, 23, 20 % respectively) (Shapas & Hilsenhoff 1976). In a Pennsylvania woodland stream its gut contents consisted of mostly animal material (~80%), followed by algae (~15%) and detritus (~5%) (Coffman et al. 1971). Mesh-sizes for fifth instars were 176 x 298 μm (Fuller & MacKay 1980a), with an increase in mesh-size for progressive instars.

1.8.8 *Hydropsyche sparna*

Hydropsyche sparna has an eastern North American distribution, occurring from Alabama to Manitoba to the treeline (Table 1.2) (Nimmo 1987). It has a wide tolerance range to environmental conditions, occurring in both cold, small, rapid streams and warm, large, slow streams (Schefter & Wiggins 1986; Schuster & Etinier 1978).

Measurements of mature larvae were not reported (Ross 1944). In Wisconsin it had a univoltine life cycle, inhabited streams containing sand (temperature maximums of 16-26 °C, widths of 0.5-21 m, currents of 0.15 to 0.75 m/s, sand to boulder substrates, slight pollution tolerance, emerging June to August) and was most common in clean woodland streams but was tolerant of agricultural runoff (Schmude & Hilsenhoff 1986). Gordon & Wallace (1975) found it in a wide range of watershed areas (0.4-843 km^2) but they were infrequent in the largest rivers (temperature 1.7-26.5 °C, dissolved oxygen 5.5-12.5 ppm). This species had a bivoltine life cycle in southern Ontario (MacKay 1979). Reported mesh-sizes for fifth instar larvae were 190 x 300 µm (Wallace 1975b) and 174 x 282 µm (Fuller & MacKay 1980a), with an increase in mesh size for progressive instars. Experimentally, *H. sparna* grew consistently faster than *H. betteni* or *H. slossonae* on all food types (detritus, diatoms, terrestrial worms) so the widespread distribution of this species may be related to its ability to more efficiently utilize a variety of food sources (Fuller & Mackay 1981). Larvae of this species are reported to be preyed upon by stoneflies (Duvall & Williams 2000).

1.8.9 *Hydropsyche ventura*

Hydropsyche ventura is limited to the Appalachian Mountains, occurring from Tennessee to Quebec (Table 1.2) (Nimmo 1987). It occurs in cold, fast unpolluted streams (Schefter & Wiggins 1986). Adult males were recorded on the west coast of Newfoundland by Schuster & Etinier (1978). Its life cycle is univoltine with low numbers of adults (1-5 per year), emerging in May near Lake Erie in Pennsylvania (Masteller & Flint 1980b), with a later emergence in June-July in the Allegheny Forest in

Pennsylvania where the sex ratio of adults was 17 male:10 female (West Fork) and 6 male:33 female (East Fork) of Hemlock Run stream (Masteller & Flint 1980a). Mesh-sizes and feeding habits were not reported in the literature.

1.9 Newfoundland Streams

Mature river systems generally follow the classical profile, with narrow, cool, headwater streams becoming broader, warmer, sediment-rich systems with reduced gradients as stream order increases. The progressive downstream changes are reflected in the stream biota. Numerous genera of Hydropsychidae are able to coexist in a single river/stream, partly because of their ability to exploit the changing conditions within a lotic system. The River Continuum Concept attempted to integrate the successive changes in physical, chemical and biological characteristics observed from headwaters to mouth in classical streams in a unified model (Vannote et al. 1980). Although there are numerous criticisms of the model, it has led to a more integrated approach to studying stream processes.

Newfoundland contains an abundance of freshwater habitats, from rivers and small streams to ponds, lakes and wetlands. Newfoundland was completely glaciated less than 15 000 years BP. This relatively recent glacial event combined with the resistant lithology cause soil here to be thin, nutrient poor and often acidic, particularly east of the Long Range Mountains where this study occurred (Damman 1983). Thus these relatively young drainage basins have poorly developed relief patterns due to the recent glaciations and resistant rock types and defining them is difficult because of the highly variable

topography. Substrates within drainage basins are poorly sorted, thus coarse and fine substrates may occur anywhere in the system. Discharge rates of Newfoundland streams are very variable due to high but irregular precipitation, occurrence of very little soil (often with low water storage capability), and highly variable gradient patterns, therefore rainfall and snow-melt greatly affect river flow (Larson & Colbo 1983). Most drainage basins in Newfoundland cover small areas (Table 1.4), which also influences water flow and nutrient levels (Larson & Colbo 1983). Newfoundland lotic systems are generally acidic with low dissolved nutrient levels and low conductivity (streams with higher pH and nutrient levels are present along the west coast) (Jamieson 1974; Jamieson 1979). Based on phosphorous concentrations, Newfoundland lentic systems are oligotrophic or mesotrophic with only a few eutrophic sites. Low phosphorous levels may limit the primary production of these water bodies. Water is frequently highly coloured which reduces light penetration and may reduce photosynthesis (Larson & Colbo 1983). Biomass of phytoplankton and zooplankton in Newfoundland lakes is generally less than that found elsewhere in North America (Campbell 1990). All these factors contribute to the low productivity of Newfoundland streams and rivers.

This results in lotic systems that differ from the classical (graded) model in terms of variable relief and substrate types. Lentic systems frequently intercept river channels as shown in Figure 1.5 (Larson & Colbo 1983). These lentic bodies interrupt the continuous transport of material down the river. Because of these factors, Newfoundland streams do not conform to the river continuum concept. Numerous wetlands throughout the Island provide sufficient storage along with ground water to allow water in almost all

stream beds to flow year round. The risk of rivers drying up in summer and freezing solid in winter is therefore low on the Island. Thus many lotic systems have conditions that can support most biota year round.

Table 1.4 Drainage basin areas in Newfoundland. From Larson & Colbo (1983).

Drainage Basin Area (km2)	*Number of Drainage Basins*
<25	4109
25-249	237
250-1299	51
1300-2599	3
> 2600	4

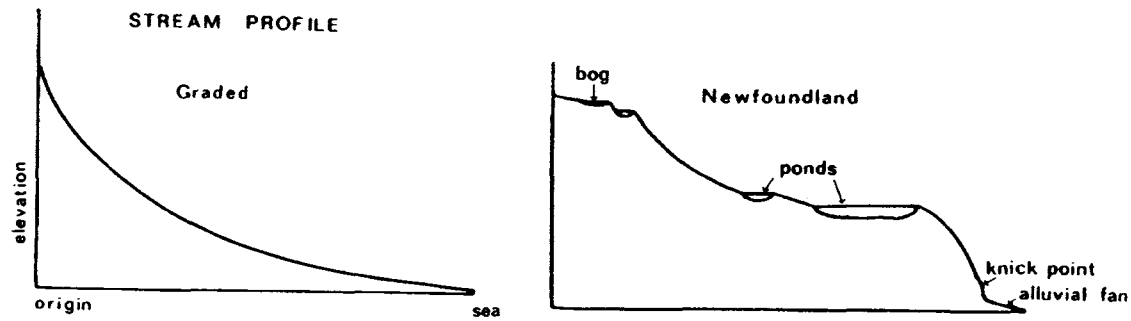

Figure 1.5 Smooth classical stream profile (left) greatly differs from a Newfoundland stream profile (right). From Larson & Colbo (1983).

1.10 Newfoundland Climate and Vegetation

The climate of Newfoundland is boreal oceanic, with high annual precipitation (900-2200 mm), cool summers (daily averages from 14°C to 16°C) and mild winters (daily averages of -4°C to -10°C) on average for the island (Banfield 1983). The cool climate, high precipitation and limited evaporation result in an ample supply of water for

freshwater habitats. Newfoundland is separated from the mainland by a minimum of 110km on the west coast and 15km on the northwest coast (2005; Lafosse 2004).

Because of its location in the cold North Atlantic Ocean, Newfoundland is exposed to high winds, which can be cold throughout the spring and summer and accompanied by fog and precipitation (Table 1.5). These high winds, together with the poor soils have contributed to the formation of many barren areas throughout the island with low relief and cool moist conditions. These areas support mosses and low-growing ericaceous vegetation or consist of various types of wetlands including blanket bogs. Rivers in barren areas contain limited dissolved nutrients because of the lack of developed vegetation and decreased run-off from nutrient rich soils (Banfield 1983). Boreal forest dominates much of the Island, particularly central and western Newfoundland. The predominant tree varieties are *Picea mariana* (black spruce), *Abies balsamea* (balsam fir), *Larix laricina* (larch), *Betula* spp. (birch) and *Populus tremuloides* (poplar). Soils in forested areas are richer and the tree cover provides shelter from wind, thus affecting the micro-climate of the area (Banfield 1983; Dodds 1997). Figure 1.6 illustrates the ecoregions of the island. This study is focused on two ecoregions: the Maritime Barrens and the South Avalon-Burin Oceanic Barrens, with some sampling also concentrated in the Central Newfoundland Forest, although a general survey of larval hydropsychid distribution was conducted across the Island.

Table 1.5 General characteristics of the ecoregions where samples were collected (Ecological Stratification Working Group 1996; Meades 1991).

Characteristics	Ecoregions of Newfoundland where samples were collected		
	Central Newfoundland Forest	Maritime Barrens	Eastern Hyper-Oceanic Barrens
Area	28 783 km^2	37 346 km^2	1 409 km^2
Mean Annual Temperature (°C)	4.5	5.5	5.5
Mean Summer Temperature (°C)	12.5	11.5	11.5
Mean Winter Temperature (°C)	-3.5	-1	-1
Annual Precipitation (mm)	1000 - 1300	1200 - 1600	1200 - 1500
Elevation (m asl)	sea level to 200	sea level to 250	sea level to 200
Summer Climate	cool	cool, foggy	cool, foggy
Winter Climate	short, cold	short, moderate	short, mild
Dominate Vegetation	black spruce	balsam fir	moss, lichen, low ericaceous shrubs
Sub Dominate Vegetation	balsam fir, birch, aspen, *Kalmia*, lichen	black spruce, tamarack, shrubs, mosses, lichen, *Kalmia*, sphagnum moss	dwarf krummholz (balsam fir)
Vegetation Pattern	Forested	Forested and Barren Areas	Barren

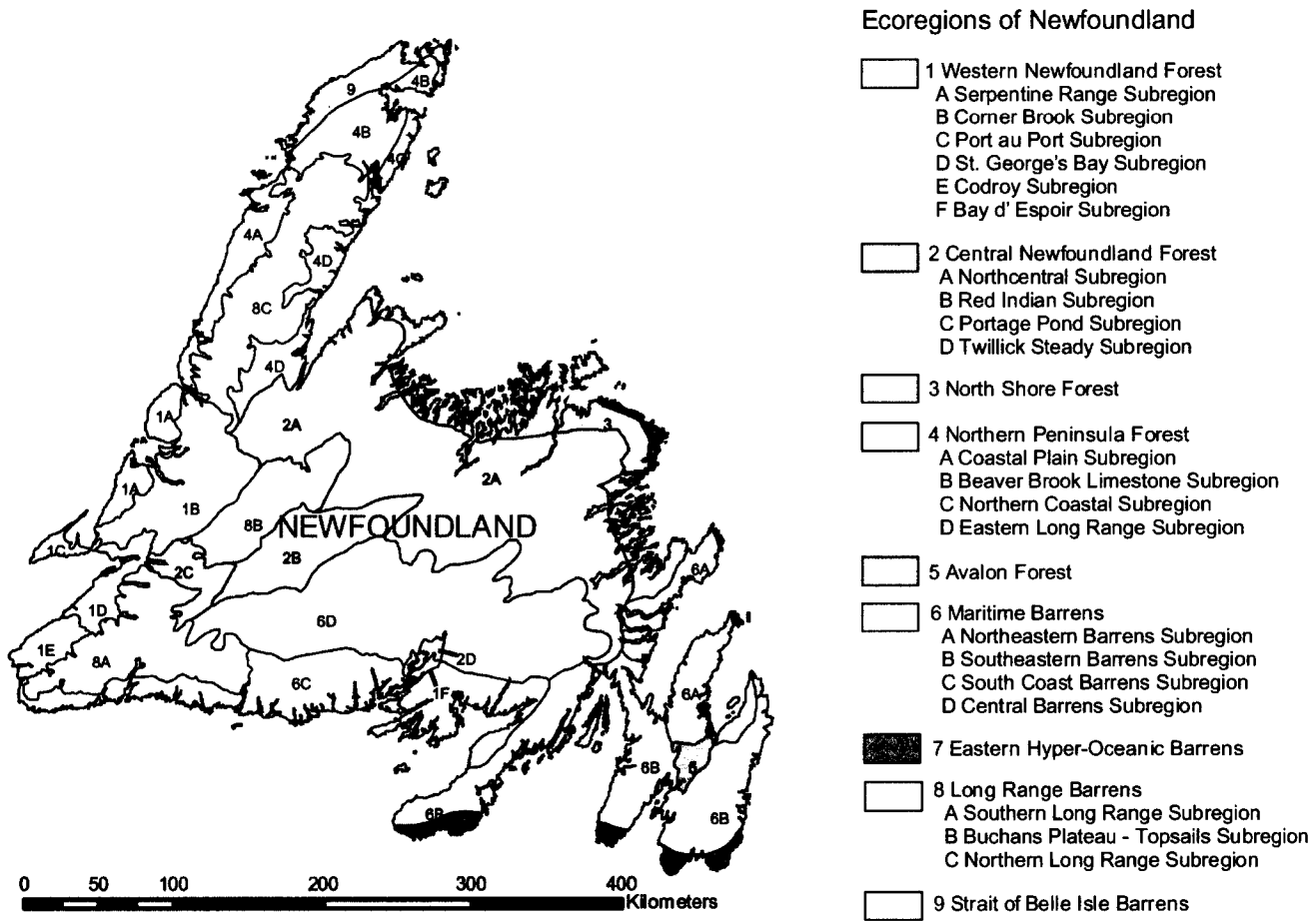

Figure 1.6 The ecoregions of Newfoundland. Used with permission (Lafosse 2004). Modified to only include the island region from a map of Newfoundland and Labrador.

2. CHAPTER 2: DISTRIBUTION OF LARVAL HYDROPSYCHIDAE IN EASTERN NEWFOUNDLAND

2.1 Introduction

Habitat is the portion of the environment occupied by a species, which Southwood (1977; 1988) suggested is a templet upon which life history traits are selected. Streams are the habitat in the current context and over the geographic range of most species, physical, chemical and biological characteristics of habitats change. Stream size (Table 1.3) and proximity to lake outlets have a strong influence on the composition and abundance of the hydropsychid community in a stream (Parker & Voshell 1983; Ross & Wallace 1982; Ross & Wallace 1983; Valett & Stanford 1987). In addition the presence/absence of forest cover influences the distribution of Hydropsychidae (Brosse et al. 2003; Collier & Smith 1997; Ross & Wallace 1982; Ross 1963; Smith et al. 2002). The known North American niches for the Newfoundland species are given in Table 1.3. Previous knowledge of Hydropsychidae in Newfoundland was derived from a broad aquatic insect survey (Larson & Colbo 1983), a Trichoptera survey (Marshall 1975) and two ecological studies on specific systems (Genge 1985; Lomond & Colbo 2000). Descriptions of the North American niches of Newfoundland species are given in section 1.8.

Newfoundland stream systems differ from classical stream systems in having a variable relief profile and lentic bodies frequently occur throughout their length (section 1.9). The combination of climate, isolation and glacial history (section 1.10) has resulted in a depauperate freshwater fauna in comparison to mainland locations where most hydropsychid studies have been conducted (section 1.7) (Wiggins 1996). One aim of this

study was to determine whether the larvae of the depauperate Newfoundland hydropsychid fauna have broader niches, in terms of the physical habitat utilized, compared to those reported on the mainland (Table 1.3), given the lack of congener species and the abundance of diverse lotic habitats on the Island (section 1.1). Habitat classification is considered in terms of three major physical characteristics of a stream: the presence/absence of a lake outlet, stream size and the surrounding vegetative patterns.

2.2 Materials and Methods

2.2.1 The Study Area

This study sampled 96 sites (Table 2.1) from a wide array of rivers and streams across Newfoundland with the greatest concentration on the Avalon Peninsula (Appendix 2 (section 10.2)). Surber sampling sites (Figure 2.1) were chosen in rivers/streams ranging in size (width at a riffle) in both forested and barren landscapes. Samples were taken at outlets and downstream sites within the same lotic system where possible. Distances at which to sample downstream were estimated from topographic maps. This estimate took into account stream size, where the distance was at least 30x the width of the stream at the outlet. To increase the data set and geographic area covered, presence/absence data of hydropsychids were compiled from rock bag and sweep net samples of previous studies (Colbo et al. 2006; Johnson 1999; Lomond 1997; Smith 2007) and are included in Table 2.1 with the general locations depicted in Figure 2.2.

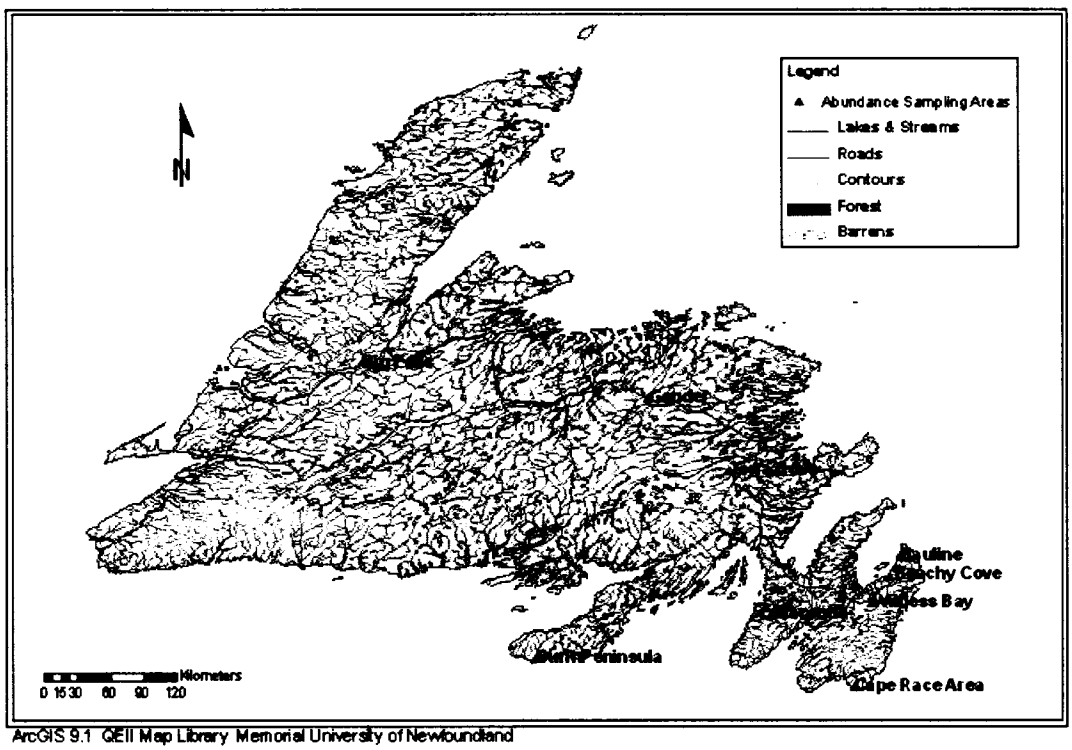

Figure 2.1 General areas where the density of Hydropsychidae were sampled.

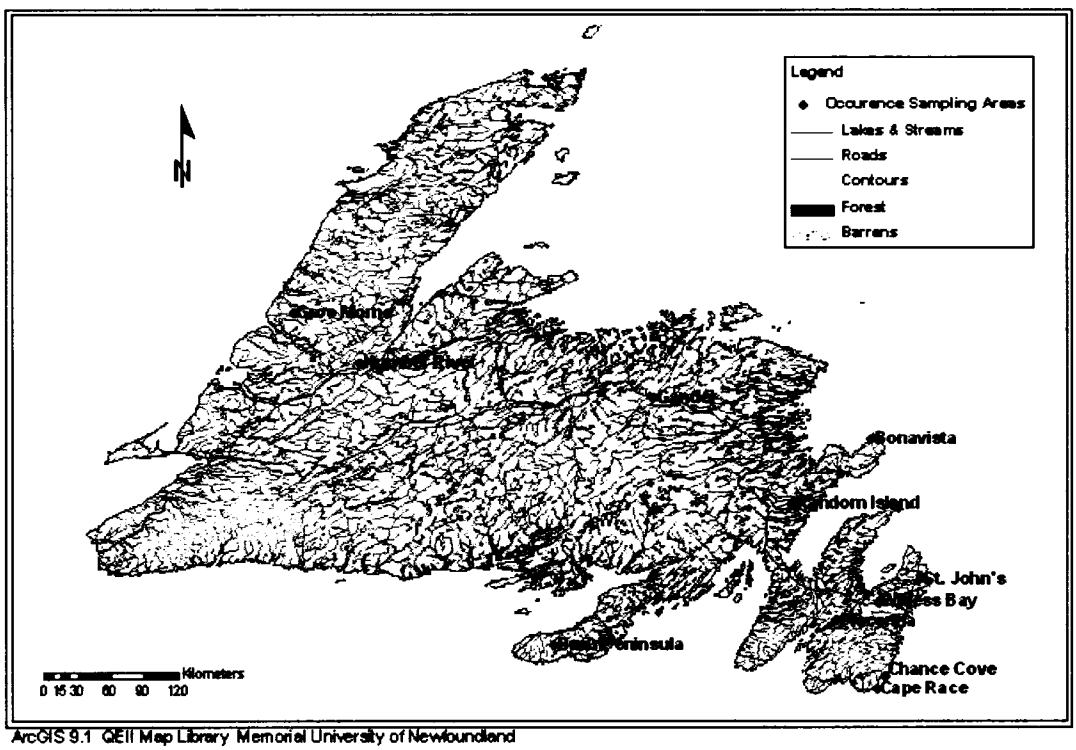

Figure 2.2 General areas where the occurrence of Hydropsychidae were sampled.

Table 2.1 Number of sites sampled by location and landscape, including data from both this and previous studies (see text).

Site Categories		Number of sites this study	Number of sites previous studies	Number of sites total
Barren	Outlet	19	4	23
	Downstream	27	6	33
	Total	46	10	56
Forested	Outlet	16	29	45
	Downstream	34	88	122
	Total	50	117	167
Total Overall		96	127	223

2.2.2 Stream Site Selection and Sampling

Samples were collected from fast-flowing riffles with stable substrates and in water depths suitable for Surber sampling, avoiding bedrock, large boulder and sand habitats. At each sample site, stream width and the range of depths within the riffle were recorded. Current velocity was estimated by timing a floating object over a 2 – 10 m distance. The pH, conductivity and temperature were measured with a YSI meter. Surrounding vegetation was classified as forested or barren, and the size composition of the substratum was estimated.

Duplicate benthic samples were collected by Surber sampler with a 0.09 m^2 quadrat and a 215 μm mesh net. Maximum length and width measurements were taken for stones larger than gravel within the Surber quadrat and their total surface area was calculated to give an estimate of the substrate area sampled. Sampling was from early fall 2001 to late spring 2002. The summer months were avoided because many larvae would have been too immature to identify. Samples were preserved in ethanol and sorted in the laboratory under a dissecting microscope. All Hydropsychidae were counted and

identified to species with the aid of keys (Marshall & Larson 1982; Schefter & Wiggins 1986; Schuster & Etinier 1978; Wiggins 1996).

2.2.3 Data Analysis

Data were entered into Microsoft Excel. Statistical analyses were carried out with Minitab version 14.1 and with SAS version 9.1. G tests with a chi-squared distribution were performed on presence/absence data to determine significant differences (α=0.05) by location, landscape and stream width categories for each species and for total hydropsychids. A Generalized Linear Model with a negative binomial distribution and type III error structure was used to determine significant differences (α=0.05) in species abundance by location, landscape and stream width categories. This was required to accurately fit a statistical model to the data as larvae had a highly clumped distribution with many zero counts. Using an ANOVA or General Linear Model was not appropriate due to the highly non-normal distribution of these data. Canonical correlation analysis was used to look for relationships between the physical/chemical variables and the hydropsychid communities. This analysis directly compares these two data matrices by reducing each to a smaller number of components and then looking for correlations between these two sets.

2.3 Results

Of the nine species of Hydropsychidae recorded in Newfoundland, only eight were collected in this study (*Hydropsyche ventura* was not collected). The occurrence data are summarized in Tables 2.2, 2.3 & 2.4 and in Figures 2.3, 2.7, 2.9 & 2.10. The density data are summarized in Tables 2.2, 2.3 & 2.5 and in Figures 2.4, 2.8, 2.11 & 2.12.

Cheumatopsyche pettiti, Hydropsyche betteni, *H. slossonae* and *H. sparna* had the highest frequency of occurrence and the greatest densities.

2.3.1 Distribution by location

A comparison of lake outlet and downstream locations showed the overall (total) frequency of occurrence of Hydropsychidae was significantly greater at outlets than at downstream sites ($p=0.002$) (Table 2.2; Figure 2.4). Three species, *C. pettiti, H. betteni* and *H. alternans*, showed a greater frequency of occurrence at outlets than at downstream sites, with differences being highly significant ($p<0.0001$). *Parapsyche apicalis* had a significantly higher frequency of occurrence ($p<0.0001$) at downstream sites compared to outlets. Four other species, *H. sparna, H. slossonae, A. ladogensis and D. modesta,* showed no significant differences in frequency of occurrence between outlets and downstream. The least often encountered species were *A. ladogensis, P. apicalis* and *D. modesta,* collected at less than 20% of the sites.

The average density for total Hydropsychidae was significantly higher at outlets than downstream sites ($p<0.0001$) (Table 2.2; Figure 2.5). Densities of *C. pettiti,* and *H. betteni* were much greater at outlets than at downstream sites ($p<0.0001$). No *D. modesta* larvae were collected at an outlet using a Surber sampler. Densities of all other species did not differ significantly by location.

Table 2.2 Comparison of the frequency of occurrence (%) and mean density per $0.1m^2$ of the Hydropsychidae by location, with standard deviation (+/-) in brackets. *** significance at $\alpha<1\%$, ** significance at $\alpha<5\%$.

	Presence			Mean Density (+/-)		
Species	at Outlets	Downstream	p	at Outlets	Downstream	p
C. pettiti	53	18	<0.0001***	222.62 (287.7)	4.87 (8.2)	<0.0001***
H. bettini	51	21	<0.0001***	135.7 (233.2)	12.22 (21.8)	<0.0001***
H. sparna	41	80	0.172	23.33 (5.60)	21.33 (4.89)	0.5255
H. slossonae	26	60	0.961	16.19 (34.3)	15.54 (22.3)	0.2879
H. alternans	21	8	<0.0001***	74.98 (76.7)	13.61 (7.1)	0.0618
A. ladogensis	4	14	0.438	2.22 (1.3)	4.87 (6.9)	0.2026
P. apicalis	1	25	<0.0001***	1.11	11.47 (12.1)	0.0979
D. modesta	1	5	0.444	none	12.44 (17.1)	na
Immature	38	63	0.025**	33.24 (69.8)	14.65 (30.4)	0.3067
Total hydropsychids	64	126	0.002***	301.8 (383.6)	30.02 (54.7)	<0.0001***
Total sites sampled	67	156		54	61	

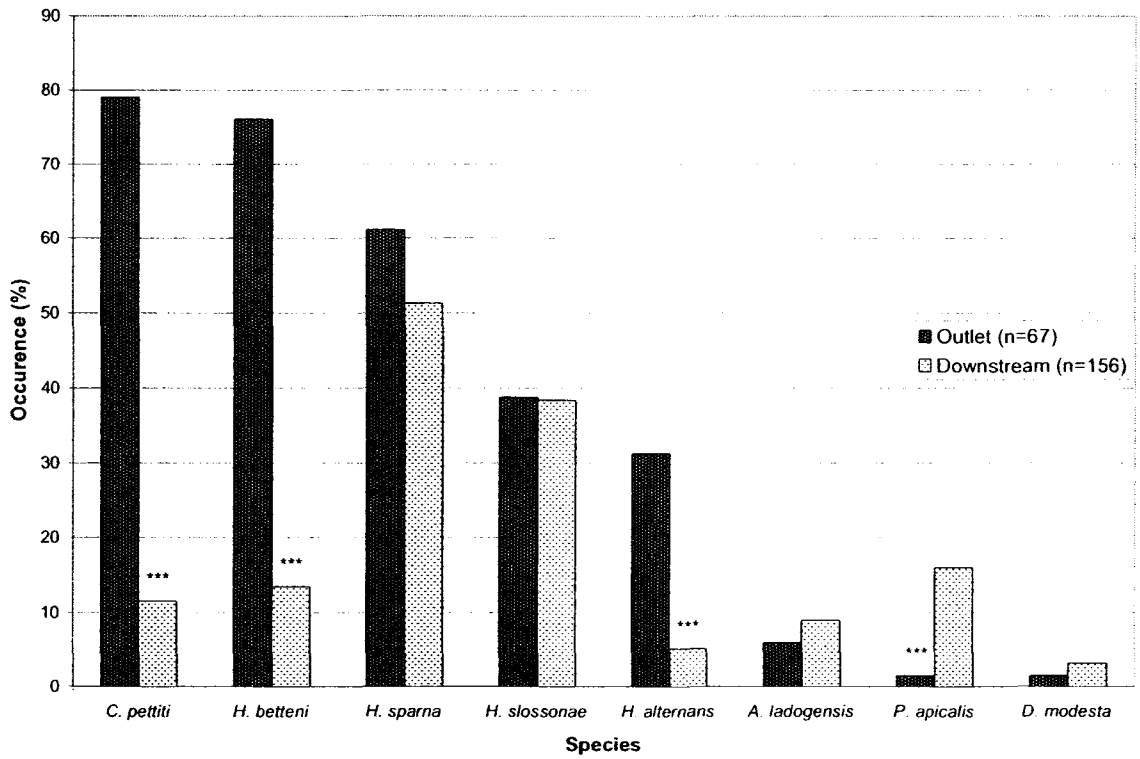

Figure 2.3 Comparison of the frequency of occurrence of Hydropsychidae as a percentage of outlets and downstream sites sampled. *** indicates significance at $\alpha<1\%$.

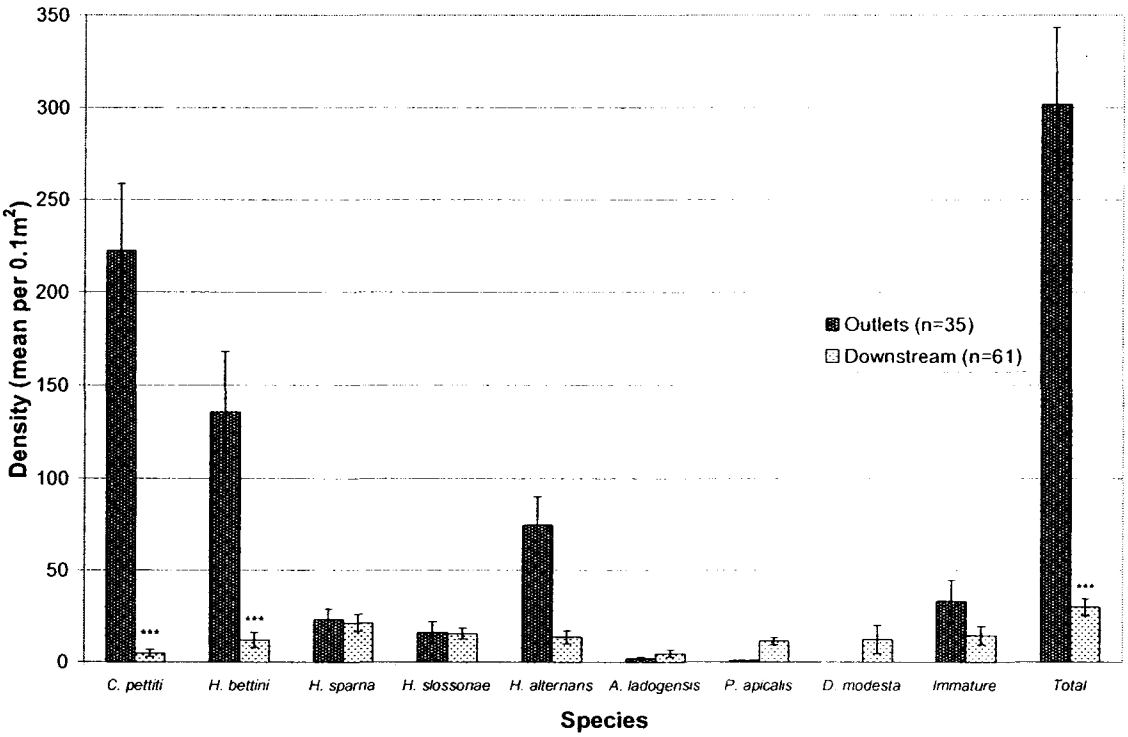

Figure 2.4 Comparison of the mean density (with standard error) of Hydropsychidae at outlets and downstream sites. *** indicates significance at $\alpha<1\%$. Only sites where Hydropsychidae were present were included. Total includes all Hydropsychidae.

Hydropsychidae outlet communities were generally distinct from downstream communities in terms of species composition and abundance. Species densities were standardized for a principal component analysis and the resulting loadings plot (Figure 2.5) showed the relative differences in species densities (along the first component), from high densities of *C. pettiti* to the rare *P. apicalis* and *D. modesta*. Considering these scores in terms of the location of the sampling site (Figure 2.6) it was evident that outlet communities were generally distinct from downstream communities (along the second component), with outlets dominated by *C. pettiti, H. betteni* and to a lesser extent *H. alternans.*

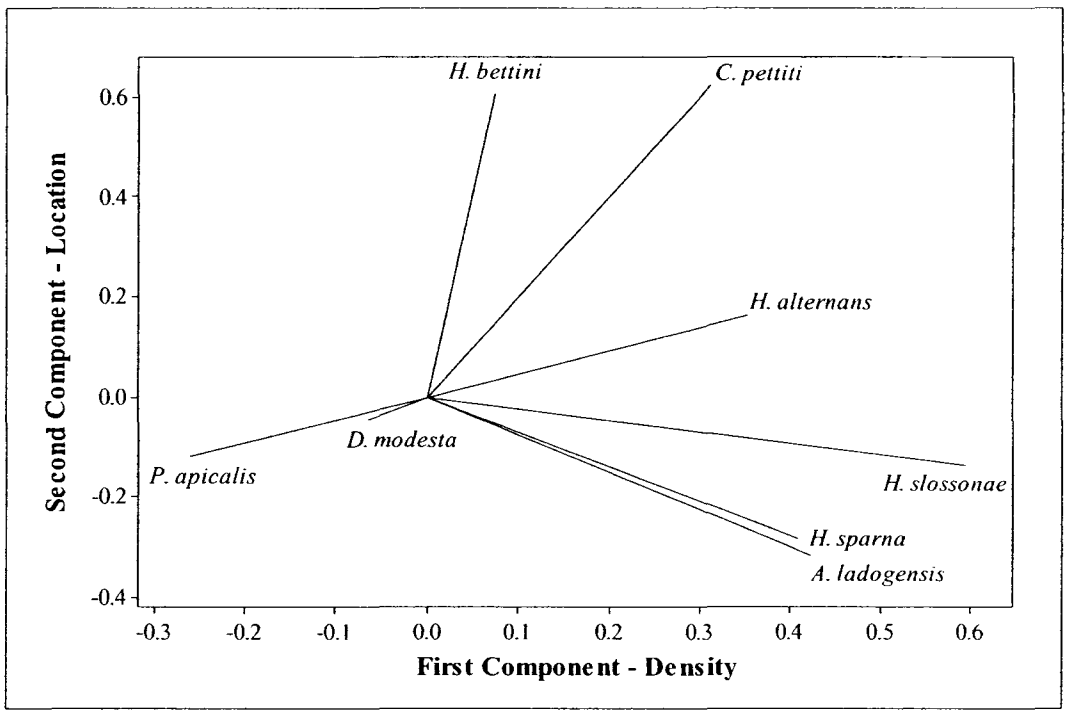

Figure 2.5 Loading plot for standardized densities of Newfoundland Hydropsychidae.

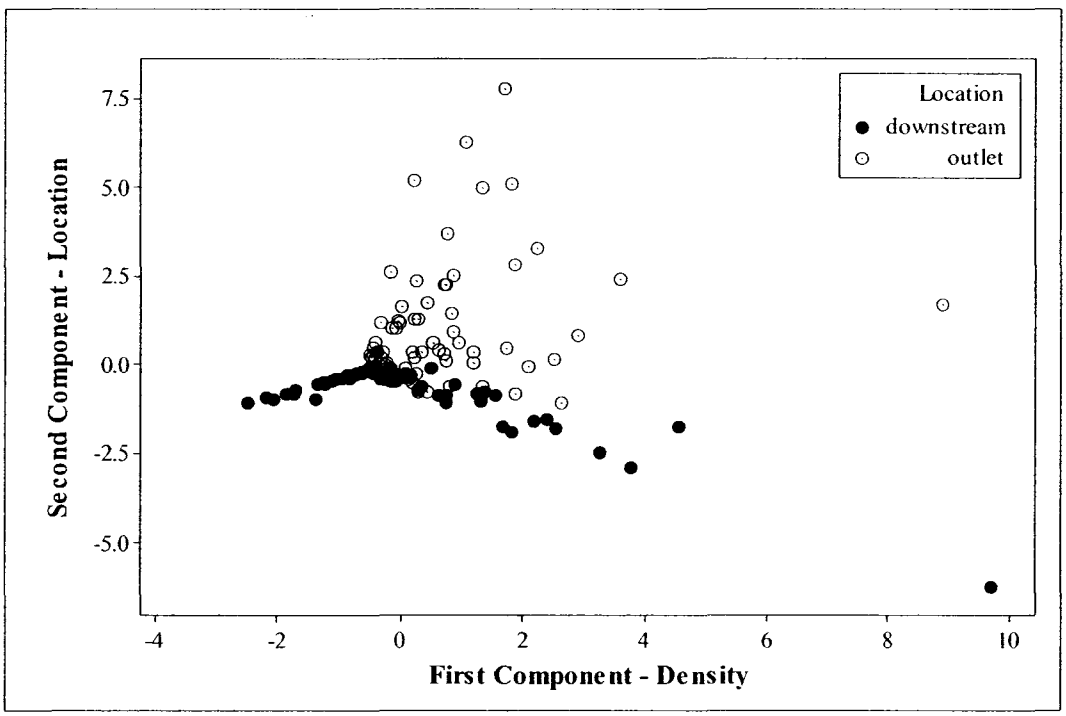

Figure 2.6 Score plot for standardized densities of Newfoundland Hydropsychidae, coded for location.

2.3.2 Distribution by landscape

All species occurred in both forested and barren sites (Table 2.3, Figure 2.7). There were no significant differences between the frequency of occurrence in forested and barren sites except for *H. slossonae* ($p=0.008$) and *A. ladogensis* ($p=0.017$) which both had higher frequencies of occurrence in barren than in forested sites.

The total density of hydropsychids was significantly higher in forested ($p=0.0014$) than in barren sites, and both *H. sparna* ($p=0.0057$) and *D. modesta* ($p=0.0333$) had significantly higher densities in forested sites. *Cheumatopsyche pettiti* ($p=0.0424$) and *H. slossonae* ($p=0.0398$) had significantly higher densities in barren sites. Density of remaining species showed no significant difference with landscape (Table 2.3, Figure 2.8).

Hydropsyche betteni ($p<0.0001$) and *H. slossonae* ($p=0.0027$) abundances showed significant interactions between landscape and location. *Hydropsyche betteni* had higher densities at forested outlets which significantly decreased downstream ($mean_{outlets} = 217.7$, $mean_{downstream} = 7.32$, $p<0.0001$), whereas densities did not differ significantly between outlets and downstream sites in barren landscapes ($mean_{outlets} = 27.53$, $mean_{downstream} = 20.56$, $p=0.5482$). *Hydropsyche slossonae* had high densities at barren outlets but the decrease downstream was not significant ($mean_{outlets} = 22.96$, $mean_{downstream} = 22.96$, $p=0.2664$), whereas in forested landscapes the opposite held true, with low densities at outlets but the downstream increase was not significant ($mean_{outlets} = 6.03$, $mean_{downstream} = 17.65$, $p=0.0656$).

Table 2.3 Comparison of the frequency of occurrence (%) and mean density per $0.1m^2$ of hydropsychids by landscape, with standard deviation (+/-) in brackets. *** significance at $\alpha<1\%$, ** significance at $\alpha<5\%$. The interaction is between location and landscape.

	Presence			Mean Density (+/-)			Interaction
Species	Forested	Barren	p	Forested	Barren	p	p
C. pettiti	50	21	0.298	150.97 (259.0)	192.0 (277.7)	0.0424**	0.0557
H. bettini	54	18	0.979	140.0 (245.3)	25.35 (37.5)	0.1026	<0.0001
H. sparna	94	27	0.295	28.27 (50.7)	14.04 (21.7)	0.0057***	0.8971
H. slossonae	56	30	0.008***	13.69 (22.1)	17.51 (31.2)	0.0398**	0.0027
H. alternans	20	9	0.44	99.06 (86.1)	40.07 (51.8)	0.0938	na
A. ladogensis	9	9	0.017**	3.33 (3.0)	4.63 (7.1)	0.4438	0.7217
P. apicalis	20	6	0.797	13.16 (13.8)	8.82 (9.5)	0.1969	na
D. modesta	5	1	0.614	19.63 (19.8)	1.67 (0.8)	0.0333**	na
Immature	79	22	0.295	29.95 (65.5)	12.74 (17.5)	0.0318**	0.0137
Total hydropsychids	140	50	0.306	169.1 (324.0)	97.20 (205.6)	0.0014***	0.2277
Total sites sampled	167	56		50	46		

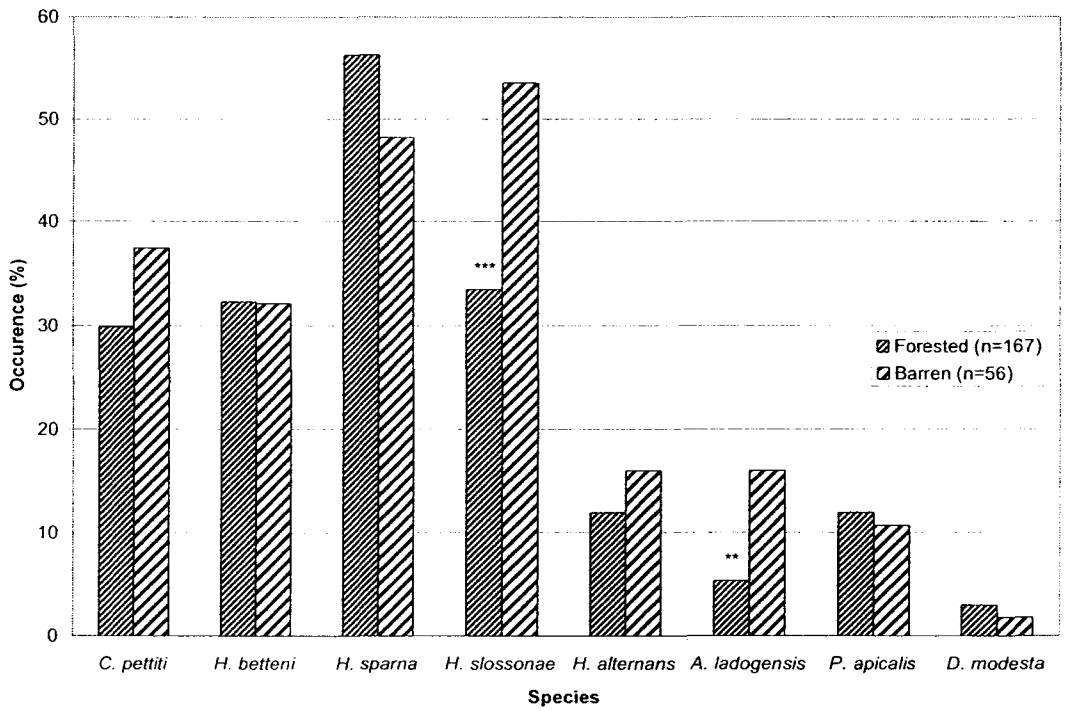

Figure 2.7 Comparison of the frequency of occurrence of Hydropsychidae as a percentage of forested and barren sites sampled. *** indicates significance at $\alpha<1\%$, ** indicates significance at $\alpha<5\%$.

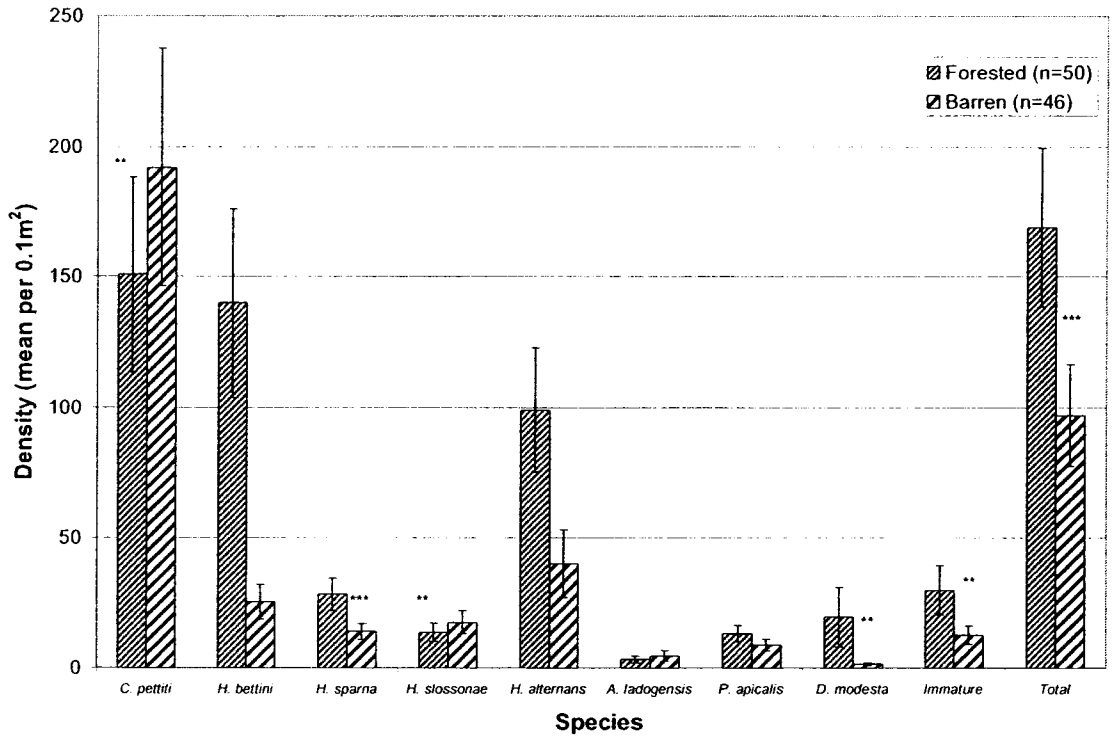

Figure 2.8 Comparison of the mean density of Hydropsychidae (with standard error) in forested and barren sites. *** indicates significance at $\alpha<1\%$, ** indicates significance at $\alpha<5\%$. Only sites where Hydropsychidae were present were included. Total includes all Hydropsychidae.

A principal component analysis allowed consideration of the community as a whole. The resulting plots did not show distinct communities based on landscape. Thus the species composition and density of the hydropsychids in forested sites were not distinct from those in barren sites.

2.3.3 Distribution by stream size

The question of whether Hydropsychidae occurrence was related to stream width was tested by grouping streams into five width categories. The frequency of occurrence of *C. pettiti, H. alternans* and *D. modesta* did not significantly differ with stream width

(Table 2.4, Figure 2.9, Figure 2.10). *Hydropsyche betteni* and *P. apicalis* were found in smaller streams, while *A. ladogensis* was more frequent in large rivers. *Hydropsyche alternans* and *H. slossonae* were rarely found in the smallest streams, with *H. slossonae* occurring in mid ranges. *Hydropsyche sparna* occurred more frequently with increasing stream size. *Diplectrona modesta* occurred rarely, found only in smaller streams. The frequency of occurrence of total hydropsychids did not differ significantly by stream width category. Changes in mean density of total hydropsychids with stream width were highly significant (p=0.0003), and this was also true for individual species except *H. slossonae* (p=0.1620) (Table 2.5, Figure 2.11, Figure 2.12).

Table 2.4 Number of streams where Hydropsychidae species were present by width category. *** indicates significance at α<1%, ** indicates significance at α<5%

	Stream width categories (m)					
Species	<1	1 to 2	2 to 5	5 to 20	>20	p
C. pettiti	11	19	23	14	4	0.196
H. betteni	17	25	18	12	0	<0.0001***
H. sparna	4	25	38	37	17	<0.0001***
H. slossonae	1	13	35	30	7	<0.0001***
H. alternans	1	4	8	10	6	0.078
A. ladogensis	0	1	4	4	9	<0.0001***
P. apicalis	12	8	5	1	0	<0.0001***
D. modesta	2	1	3	0	0	0.602
Total hydropsychids	23	43	54	51	19	0.198
Total sites sampled	29	46	68	59	21	

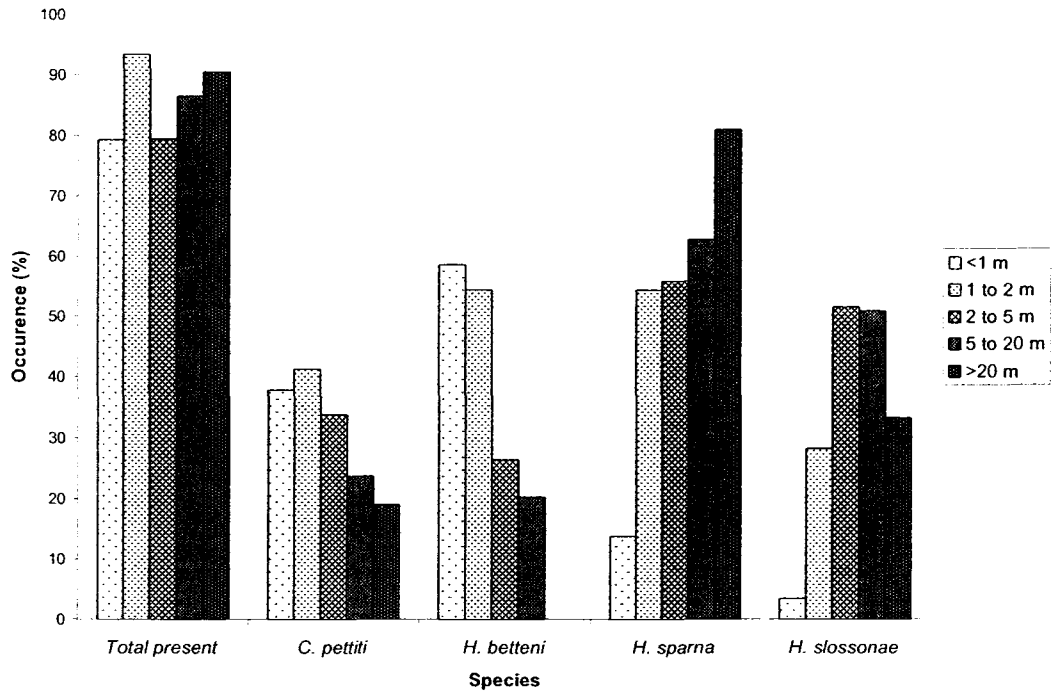

Figure 2.9 Comparison of the frequency of occurrence as a percentage of the total sites sampled of five commonly occurring hydropsychid species by stream width category (m).

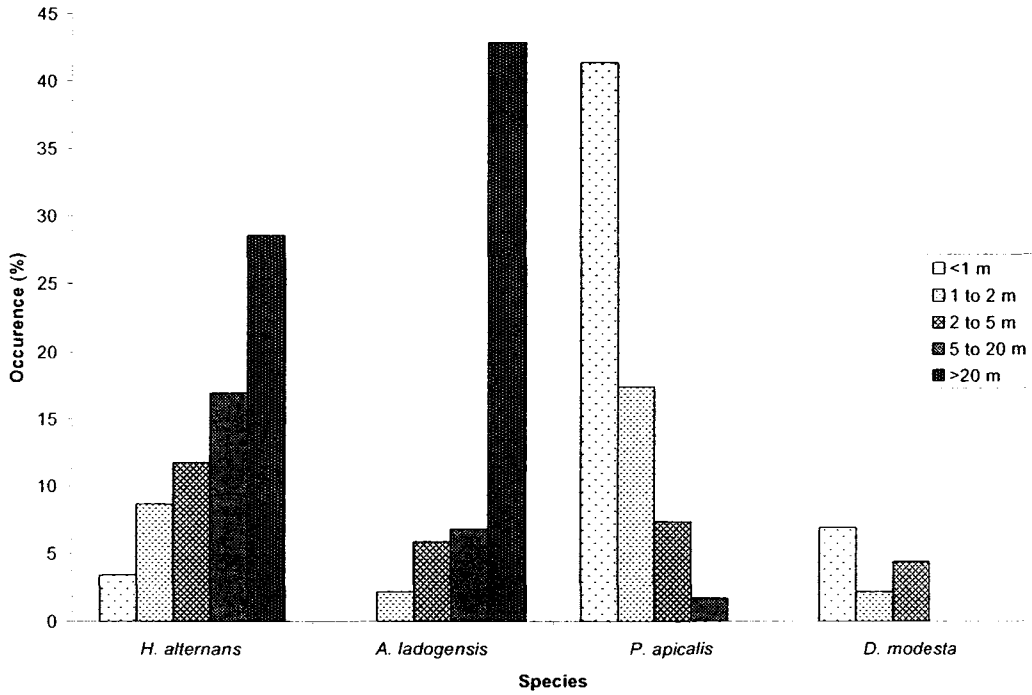

Figure 2.10 Comparison of the four species with lower frequencies of occurrence as a percentage of the total sites sampled by stream width category (m).

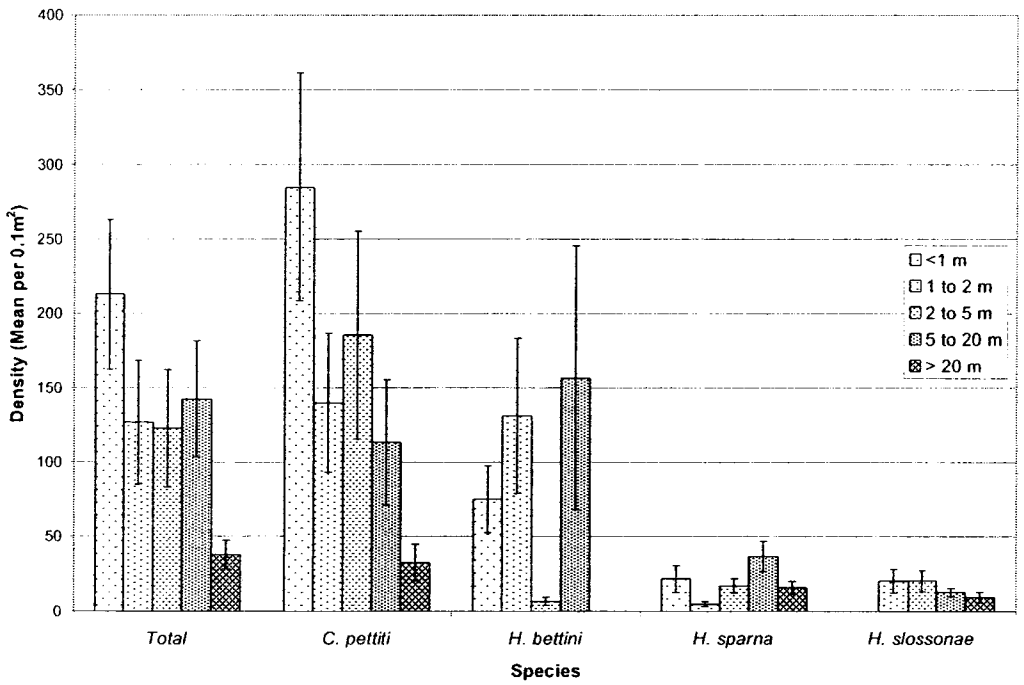

Figure 2.11 Comparison of the mean density per $0.1m^2$ (with standard error) by stream width category for total hydropsychids and for the four hydropsychid species with the highest densities.

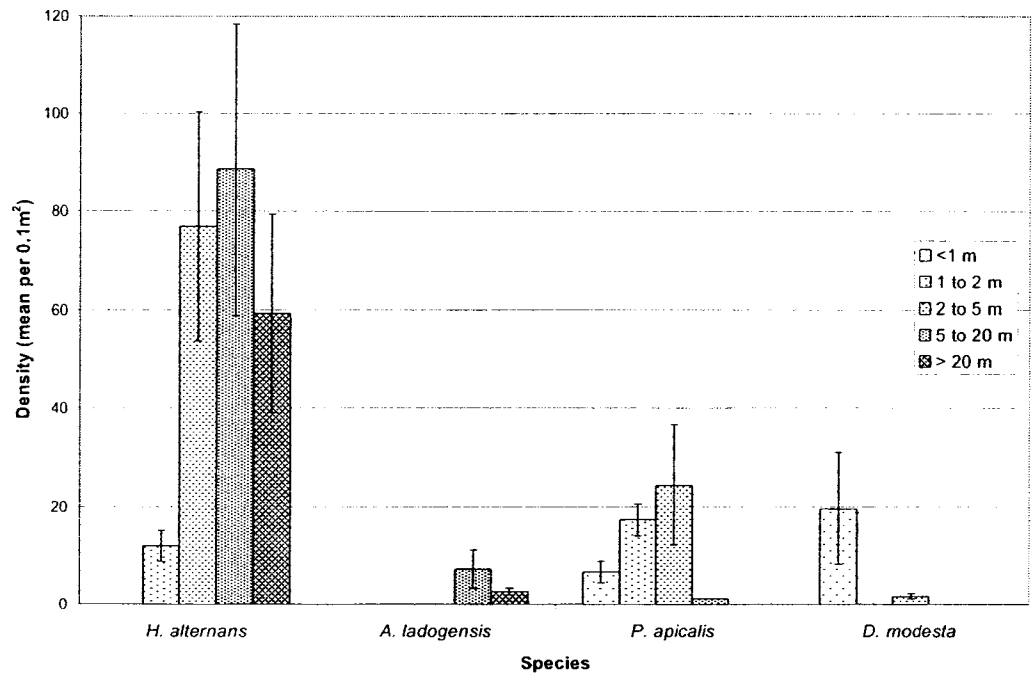

Figure 2.12 Comparison of the mean density per $0.1m^2$ (with standard error) by stream width category for four hydropsychid species with lower densities.

Table 2.5 Mean density and standard deviation (+/-) of species of Hydropsychidae with categories of stream width (m). *** indicates significance difference at $\alpha<1\%$, ** indicates significance difference at $\alpha<5\%$ amont categories per species.

	Stream width categories (m)															
	<1			1 to 2			2 to 5			5 to 20			>20			
Species	n	mean	+/-	n	mean	+/-	n	mean	+/-	n	mean	+/-	n	mean	+/-	p
C. pettiti	22	284.7	357.8	14	140.0	175.1	18	185.5	296.6	22	113.3	198.2	9	32.59	36.3	0.0313**
H. bettini	31	74.95	22.55	25	131.2	52.30	12	6.67	2.73	10	156.8	88.72	0	*	*	0.0002***
H. sparna	8	21.81	25.3	17	5.03	6.5	24	17.13	23.2	39	36.75	62.6	29	15.98	21.6	<0.0001***
H. slossonae	0	*	*	9	20.37	23.7	32	20.45	39.4	37	12.82	17.5	13	9.57	12.9	0.1620
H. alternans	0	*	*	5	12.00	7.1	7	76.98	61.7	11	88.59	98.8	6	59.26	49.4	0.0341**
A. ladogensis	0	*	*	0	*	*	0	*	*	6	7.22	9.7	11	2.63	2.2	0.0323**
P. apicalis	20	6.61	10.1	12	17.41	11.3	2	24.44	17.3	1	1.11	*	0	*	*	0.0068***
D. modesta	3	19.63	19.8	0	*	*	2	1.67	0.8	0	*	*	0	*	*	0.0333**
Immature	14	60.24	103.1	17	11.11	12.1	11	7.17	11.5	23	24.59	43.2	8	7.78	10.1	<0.0001***
Total hydropsychids	46	213.0	341.8	47	126.9	285.6	42	122.7	257.0	53	142.5	284.6	35	37.94	55.3	0.0003***

2.3.4 Relation of species abundance to physical and chemical variables

General observations were made about species' retreat construction that should be noted. *Hydropsyche alternans* usually occurred on fine gravel substrates, sometimes with coarse sand, which were incorporated into its retreats and perhaps the availability of this material partially influences its distribution. *Hydropsyche betteni* was observed to burrow into organic material surrounding embedded substrates (M. Colbo, pers. comm.), an ability that may allow it to exploit a habitat unavailable to other species.

The relationship between physical and chemical variables (width, depth, substrate area, pH, conductivity and temperature) and the hydropsychid community was examined by canonical correlation analysis between the physical/chemical variables and the densities of the eight species. The physical/chemical variables were reduced to two axes as was the hydropsychid community structure and then relationships between these two sets of variables were investigated using canonical correlation (Table 2.6). The first two canonical variables accounted for most of the variability in the data (86.08%), and the first variable was highly significant ($p<0.0001$). The density of *A. ladogensis* was positively correlated with the width and depth of a stream (correlation coefficient of 0.4791). The second canonical correlation showed a negative relationship between width and substrate area, which had a weak correlation with *H. sparna* (0.2400) and *D.* modesta (0.2312). The other species had very weak relationships with the measured variables (Table 2.6).

Table 2.6 Correlations for the PCA analysis among the physical/chemical variables and among the species densities with their canonical variables and then the correlation between these two groups.

Physical/Chemical	PCA of physical/chemical			PCA of Species		Canonical Correlation	
Variables	axis 1	axis 2	Species	axis 1	axis 2	phys/chem 1	phys/chem2
Width	0.8132	-0.3206	*A. ladogensis*	0.7793	-0.272	0.4791	-0.1171
Depth	0.9356	-0.1412	*C. pettiti*	-0.1051	0.466	-0.0646	0.2006
velocity	0.1032	0.0449	*D. modesta*	0.0.312	0.5371	0.0192	0.2312
substrate number	-0.031	-0.0335	*H. alternans*	0.4522	0.2294	0.2780	0.0988
total substrate area	0.2574	0.5375	*H. betteni*	-0.11	0.1398	-0.0676	0.0602
pH	-0.0196	-0.0641	*H. slossonae*	-0.0054	0.2726	-0.0033	0.1174
conductivity	-0.1865	-0.0162	*H. sparna*	0.4197	0.5574	0.2580	0.2400
temperature	0.5084	0.5495	*P. apicalis*	-0.2237	-0.1548	-0.1376	-0.0666

2.4 Discussion

2.4.1 Effect of location

Three of the eight species, *C. pettiti, H. betteni* and *H. alternans* occurred more frequently and in greater abundances at outlets, creating distinct outlet and downstream communities in Newfoundland streams (Figure 2.3). The reduced number of hydropsychid species in Newfoundland was postulated to allow species to expand their use of physical habitat. This was not the case. Outlets are known to influence communities (Parker & Voshell 1983; Ross & Wallace 1982; Ross & Wallace 1983; Valett & Stanford 1987), possibly because of the increase in nutrients for filter feeders emanating from the lake (Richardson & MacKay 1991). Genge (1985) studied the caddisfly community in a single Newfoundland stream and found similar results, with *C. pettiti* and *H. betteni* in higher densities at the outlet and *H. sparna* and *H. slossonae* being denser downstream. Thus, despite the depauperate fauna here, there is a well defined lake outlet and downstream community.

Cheumatopsyche pettiti was restricted to outlets in Newfoundland, but not elsewhere (Gordon & Wallace 1975). This species prefers warm, enriched waters including downstream sites (Kondratieff et al. 1997) but was rare far from an outlet in this study. This could be influenced by temperature, with outlets presumably being a warmer habitat. *Hydropsyche betteni* was also mainly an outlet species in Newfoundland, whereas it has been found at high densities in downstream sites elsewhere (Ross & Wallace 1983). *Hydropsyche alternans* has a broad habitat range (Schefter & Wiggins 1986), but here it mainly occurred at outlets where its frequency of occurrence reached only about 30% (Figure 2.3). *Hydropsyche sparna* was the most frequently encountered

species (Table 2.2) and has a very broad habitat range (Schefter & Wiggins 1986) which was supported here where it showed no difference in occurrence between outlets or downstream sites. Because this species is a generalist feeder (Fuller & Mackay 1981) it may be able to exploit a wide range of habitat types. *Hydropsyche slossonae* was the second most frequently encountered species here. It had a broad habitat range with a similar occurrence at outlets and downstream sites (Figure 2.3), which is similar to a Minnesota stream (MacKay 1986).

The low frequency of occurrence of *A. ladogensis, P. apicalis* and *D. modesta* was generally at downstream sites and is similar to their occurrence elsewhere (Brittain & Bildeng 1995; Flint 1961; Haefner & Wallace 1981; Ross & Wallace 1982; Ross & Wallace 1983). Although *A. ladogensis* was not common at lake outlets here, it was abundant at a 100 m wide outlet in Labrador (LGL Limited 1999).

Hydropsyche ventura was not found, although it was previously collected from a 3 m wide stream in western Newfoundland (Schuster & Etinier 1978). This site was sampled for larvae in early September, but only *P. apicalis* and *H. sparna* were found. All of the other eight species of hydropsychids were collected throughout the Island. Therefore regional processes do not appear to influence the distribution of the remaining eight Hydropsychidae species on the Island.

Diplectrona modesta was rarely encountered but was found in a wide range of habitat types. It was found at sites in the Barking Kettle - Broad Cove stream system, where it was found in the 1970s (Marshall & Larson 1982). *Diplectrona modesta* was found in very low numbers ($1.6/m^2$) where Barking Kettle (0.5 m wide) joins Broad Cove

stream (3.5 m wide) in this study. This species was only found at two other sites and in low densities: Great Pond, a 2m wide stream approximately 13 kms away from Barking Kettle, and in a tributary to Finnies Pond (3.8 m wide, approximately 28 kms west). This sparse distribution might be the result of adults accidentally blown in from the mainland, which is known to occur for several insect species (Compton 2002; Gatehouse 1997). However, the persistence of a population in the Barking Kettle – Broad Cove stream system for over 30 years would suggest viable populations occur here and the limited distribution is due to other factors.

The greatest density of total hydropsychids was at lake outlets in all streams quantitatively sampled (Table 2.2, Figure 2.4). This finding is in agreement with several previous studies (Hoffsten 1999; Morin & Harper 1986; Richardson & MacKay 1991). At outlets flow is laminar and relatively constant, even during periods of low water levels. Sediment levels are low because the lake is acting as a sediment sink and thus sediment scouring of the stream bottom is greatly reduced. Such stable conditions favour dense hydropsychid populations. Competition is minimized by the abundance of nutritional resources so larvae are able to co-exist in close proximity to each other (Richardson & MacKay 1991). Larval retreats in this study were observed on top of each other at some outlets. In laboratory experiments with high larval densities and scarce resources, larvae attacked their close neighbours until one abandoned its retreat (Matczak & MacKay 1990). This suggests that direct competition for food could affect population densities, so outlets here are postulated to have an abundant food supply.

Seston issuing from lakes into rivers and its effect on invertebrates has been discussed by several authors (Hoffsten 1999; Morin & Harper 1986; Oswood 1979; Perry & Sheldon 2005; Richardson & MacKay 1991; Vadeboncoeur 1994). One outlet species, *H. betteni* is carnivorous (Coffman et al. 1971; Fuller & MacKay 1980a), suggesting its abundance is attributable to high zooplankton levels. Genge (1985) found this species to consume animal material at a Newfoundland outlet. *Hydropsyche slossonae*, *H. sparna* and *A. ladogensis* showed no significant difference in abundance between outlet and downstream sites and thus are not directly dependent on outlet food resources, which is also true for *D. modesta* and *P. apicalis* which rarely occurred at outlets. *Diplectrona modesta* may rarely occur at outlets as it feeds on detrital particles which are more abundant downstream than at outlets (Minshall 1967). *Parapsyche apicalis* is a cool stream species (Flint 1961) and elevated temperatures at outlets may be lethal. *Arctopsyche ladogensis* rarely occurred at outlets and it appears the frequency of occurrence and population densities in Newfoundland were lower than would be predicted based on other studies (Brittain & Bildeng 1995).

Lakes disrupt the downstream movement of material and so Newfoundland streams differ from the classical pattern predicted by the River Continuum Concept (Vannote et al. 1980). A more appropriate model for Newfoundland streams may be the Hierarchical Patch Dynamics (HPD) hypothesis of Wu & Loucks (1995) and applied to streams by Poole (2002). This more flexible and robust model would allow for the dynamic patterns evident in Newfoundland streams (section 1.1, section 1.9).

2.4.2 Effect of landscape

This study was the first to compare sites from forested and barren landscapes for these taxa within the same region. All species occurred in both forested and barren drainage basins indicating that colonization was independent of tree cover. *Arctopsyche ladogensis* and *H. slossonae* had higher frequencies of occurrence in barren landscapes than in forested sites. Occurrence of all other species exhibited no significant difference between landscapes (Table 2.3, Figure 2.7).

The abundance of total hydropsychids, *H. sparna* and *D. modesta* were significantly higher in forested sites than barren sites and the opposite was true for *C. pettiti* and *H. slossonae* (Table 2.3, Figure 2.8). Forested streams generally have more allochthonous input from the surrounding vegetation which *H. sparna* is able to exploit (Fuller & Mackay 1981). While lack of shading in barren streams will promote growth of autochthonous periphyton and reduced terrestrial biomass will reduce terrestrial input. In general, *H. slossonae* and *H. sparna* frequently occurred together and are thought to avoid competition by partitioning food resources (Begon et al. 1996; Fairchild & Holomuzki 2002; Fuller & MacKay 1980a).

Nutrient spiraling may also be contributing to changes in community composition. Streams in barren landscapes may have less allochthonous material because of the lack of leaf fall and so nutrient spiraling dynamics will be different than in forested landscapes (Hynes 1975). A decline in food levels can lead to longer retention time of food by an organism in order to increase absorption of nutrients and thus the spiraling rate of a particle will decrease (Merritt et al. 1984). Species dependent on spiraling would thus have lower densities at barren downstream sites.

2.4.3 Effect of stream size

The range of stream sizes colonized by Hydropsychidae species occurring in Newfoundland resembled that of the mainland. Although species diversity was reduced, this did not translate into utilization of a broader range of stream sizes. However, stream size did influence species composition and density. Lower hydropsychid densities in larger streams here may have resulted from generally lower concentrations of allochthonous inputs and export of more inorganic sediment than smaller streams (Naiman 1983; Naiman et al. 1987).

Stream habitat use by *A. ladogensis* and *P. apicalis* agreed with that outlined by Flint (1961) for North America in general. *Diplectrona modesta* was rare, occurring in smaller streams, which differs from the broad range of stream sizes inhabited elsewhere (Gordon & Wallace 1975; Masteller & Flint 1979). *Cheumatopsyche pettiti* occurred across the range of stream sizes sampled. This genus is found in a wide range of stream sizes elsewhere (Gordon 1974), but larvae were not identified to species so further comparisons can not be determined(Ross 1944). *Hydropsyche alternans* were more frequently found in large streams but there was generally a low frequency of occurrence. However, its density was highest in large streams, exceeding 80 individuals per $0.1m^2$ and at a 100 m wide outlet in Labrador its abundance (mean=125 ± 20 per 3 L rock bag) was on par with that of *A. ladogensis* (mean=181 ± 25 per 3 L rock bag) (LGL Limited 1999), which supports an affinity for larger streams. *Hydropsyche betteni* occurred in smaller streams here and elsewhere (Schuster & Etinier 1978). *Hydropsyche slossonae* was found in mid to large streams here and Shuster & Etiner (1978) reported them from larger streams elsewhere. *Hydropsyche sparna* occurred with increasing frequency as

stream size increased and its occurrence has been recorded in a broad range of stream sizes in North America (Schefter & Wiggins 1986; Schuster & Etinier 1978).

2.4.4 Discussion of assessing hydropsychid density

Determining hydropsychid population size in a stream reach is extremely difficult and not attempted here. Rather the goal was to determine the relative density of species from similar habitat types in different reaches within and among streams. There was not a consistent trend between the frequency of occurrence and mean density among stream sizes for any of the species.

Arctopsyche ladogensis, *P. apicalis* and *D. modesta* occurred widely but always at very low densities here. *Arctopsyche ladogensis* occurs in large rivers with deep waters and large substrates (Flint 1961) which were difficult to sample and not sampled here. As noted above, LGL Limited (1999) reported high densities in a large Labrador river. Low densities of *P. apicalis* may reflect a lower productivity of the small, cool streams it inhabits. *Diplectrona modesta* was rarely found here and usually at very densities (~2 per m^2) with the highest mean density of 29 per m^2 in Barking Kettle. This is far below a maximum mean density of 136.7/m^2 in a 2m wide woodland stream in Tennessee (Cushman et al. 1975).

Low abundances of hydropsychids, in general, in many Newfoundland streams may reflect their poor productivity. Most Newfoundland streams are oligotrophic (Larson & Colbo 1983), so stream productivity may not support large population sizes as food resources may be limiting. Low stream productivity may lead to strong competition for food and consequently influence the diversity of species at a given site.

Previous studies indicated long emergence periods for adults of species considered here (Table 1.3). During the current study, pupae were observed from June through August, confirming a long period of adult emergence that could be reflective of low resource productivity. For hydropsychids with low densities this long emergence time could adversely affect adult mating success and the tendency for males to emerge before females may exacerbate this effect (Flannagan & Lawler 1972). In southern Ontario, for example, *P. apicalis* had a long emergence time with an unbalanced sex ratio in the few adults collected at a given time (Singh et al. 2005). The sex ratio of adults also differs with daily time of emergence. Female *C. pettiti* were found to emerge earlier in the evening than males in a Kentucky stream (Resh et al. 1975). If adults cannot find a mate because of low population densities, long emergence times and unbalanced sex ratios then reproductive output may be reduced.

The broad geographic distribution of these caddisflies in Newfoundland may reflect the dispersal abilities of gravid females ovipositing in non-natal streams rather than the viability of populations in streams. Thus the presence and density of larvae at a given site could result from ovipositing females from another site. However, Hydropsychidae are thought to be weak fliers (Nimmo 2003), rarely traveling more than 5 km from a natal stream (Kovats et al. 1996). Although adults can be carried by wind (Wolf et al. 1986). Thus constant recolonization may reduce the ability to determine prime habitat occurance from survey data.

Benthic invertebrates have highly contagious distributions, thus obtaining an accurate assessment of invertebrate population density in a stream by Surber sampling is

not practical because a large number of samples are needed to accurately estimate a population with a patchy distribution (Resh 1979). In this study, similar habitats at each site were sampled to minimize variability caused by substrate type and water velocity. Cobble sized substrates were sampled when possible because hydropsychids require stable substrates on which to build their fixed retreats (Cardinale et al. 2004). They occupy the porous areas within the stable substrate which can extend as much as 50 cm below the substrate surface (Williams & Hynes 1974). The vertical distribution within the substrate adds to the difficulty in accurately estimating their densities.

Penetration of hydropsychids into substrates was observed here at several sites in the loose cobble substrate with larvae found 10 cm or more below the surface. Water flow was present, although velocities would have been low. *Cheumatopsyche pettiti* was frequently found in this type of habitat, with densities remaining high with increased substrate depth; however smaller instars were found deeper in the substrate and larger instars were found near the surface. Thus the substrate may be a refuge from predators for younger instars, or competition for space with larger cannibalistic instars may produce this separation (Willis & Hendricks 1992). In addition, younger instars with finer nets (Wallace & Merritt 1980) can possibly obtain adequate nutrition from smaller detrital particles at depth in the substrate. A vertical division of space by life stage was found by Rutherford & MacKay (1985) with larval stages at greater densities in upper layers and pupae in middle reaches. Intra-guild competition may also partition the space as Williams & Hynes (1974) also never found *Hydropsyche* at depth in the substrate but

found *Cheumatopsyche pettiti* at depths up to ~50cm, possibly because of the wide tolerance range of *Cheumatopsyche* to physical conditions.

2.5 Conclusion

All eight species of hydropsychids collected in this study were widespread throughout the Island with no broad regional differences. All species were also found in streams surrounded by both forested and barren landscapes. Stream size affected hydropsychid distribution as did the presence of lake outlets, possibly because of differences in nutrient concentrations and water temperatures. Overall, densities were elevated at outlets and were greater in streams surrounded by forested landscapes, although this differed among species. Weak correlations between the physical/chemical variables measured and the species density indicated that these factors were not strong influences on the ecological make up of the hydropsychid community. Although the number of hydropsychid species is greatly reduced in Newfoundland, there appeared to be no broadening of their resource exploitation. Therefore the hypothesized reduced spatial and nutritional competition between species because of the impoverished fauna did not translate into an expanded habitat range in Newfoundland. Questions arising from this study are: can patterns in hydropsychid distribution and density be related to food availability; and is it possible to model the effect of lake outlets across streams of different sizes in forested and barren landscapes?

3. CHAPTER 3: THE INFLUENCE OF TEMPERATURE AND SESTON ON THE DISTRIBUTION OF LARVAL HYDROPSYCHIDAE IN EASTERN NEWFOUNDLAND

3.1 Introduction

In this chapter the quantities of seston are compared across categorical stream sizes (section 2.3.3) to determine relationships between hydropsychid densities and quantities of phytoplankton and zooplankton in stream seston, in relation to outlets, local vegetation patterns and stream size. Hydropsychidae larvae occur in high densities at the outlets of lakes (Spence & Hynes 1971) which has been attributed to (Parker & Voshell 1983; Ross & Wallace 1983; Valett & Stanford 1987)the presence of high quality food, stable substrates and constant water flow (Cushing 1963; Georgian & Thorp 1992; Valett & Stanford 1987). Predominant sources of food at outlets are lentic phytoplankton and zooplankton in the seston, while further downstream allochthonous inputs become increasingly important (Richardson & MacKay 1991; Vannote et al. 1980). Allochthonous inputs are of terrestrial origin and so the riparian vegetation will affect the quantity and/or quality of these inputs (Drake 1984; Merritt & Cummins 1996). Two broad types of vegetation occur in drainage basins on the Avalon Peninsula of Newfoundland: 1) forested, dominated by black spruce and balsam fir; and 2) barren, dominated by low growing shrubs, herbs, grass, sedges and mosses (Table 1.5). Stream size was shown in Chapter 2 to influence the occurrence of Hydropsychidae (Table 2.4), which is also true elsewhere (Schefter & Wiggins 1986; Schuster & Etinier 1978; Wiggins 1996).

Water temperatures at outlets are elevated compared to downstream because the lake absorbs solar radiation (Giller & Malmqvist 1998) and temperature is known to influence the occurrence of Hydropsychidae (Table 1.3) (Hauer & Stanford 1982a). Thus temperature was measured in this study at outlets and downstream sites in both forested and barren landscapes. Surrounding vegetation patterns can influence water temperature by shading and barren areas tend to have cool winds (Ecological Stratification Working Group 1996; Meades 1991). It is known that one Newfoundland species, *P. apicalis* is limited to cooler streams (Flint 1961).

3.2 Materials and Methods

3.2.1 The Study Area & Benthic Sampling

Duplicate Surber samples were take from 27 sites on the Avalon Peninsula of Newfoundland in the fall of 2002 (Table 3.1, Figure 3.1, (Appendix 2 (section 10.2))) and physical characteristics were recorded. Detailed sampling methods are given in section 2.2.2. Effect of stream width was tested by grouping streams into five width categories (see section 2.3.3).

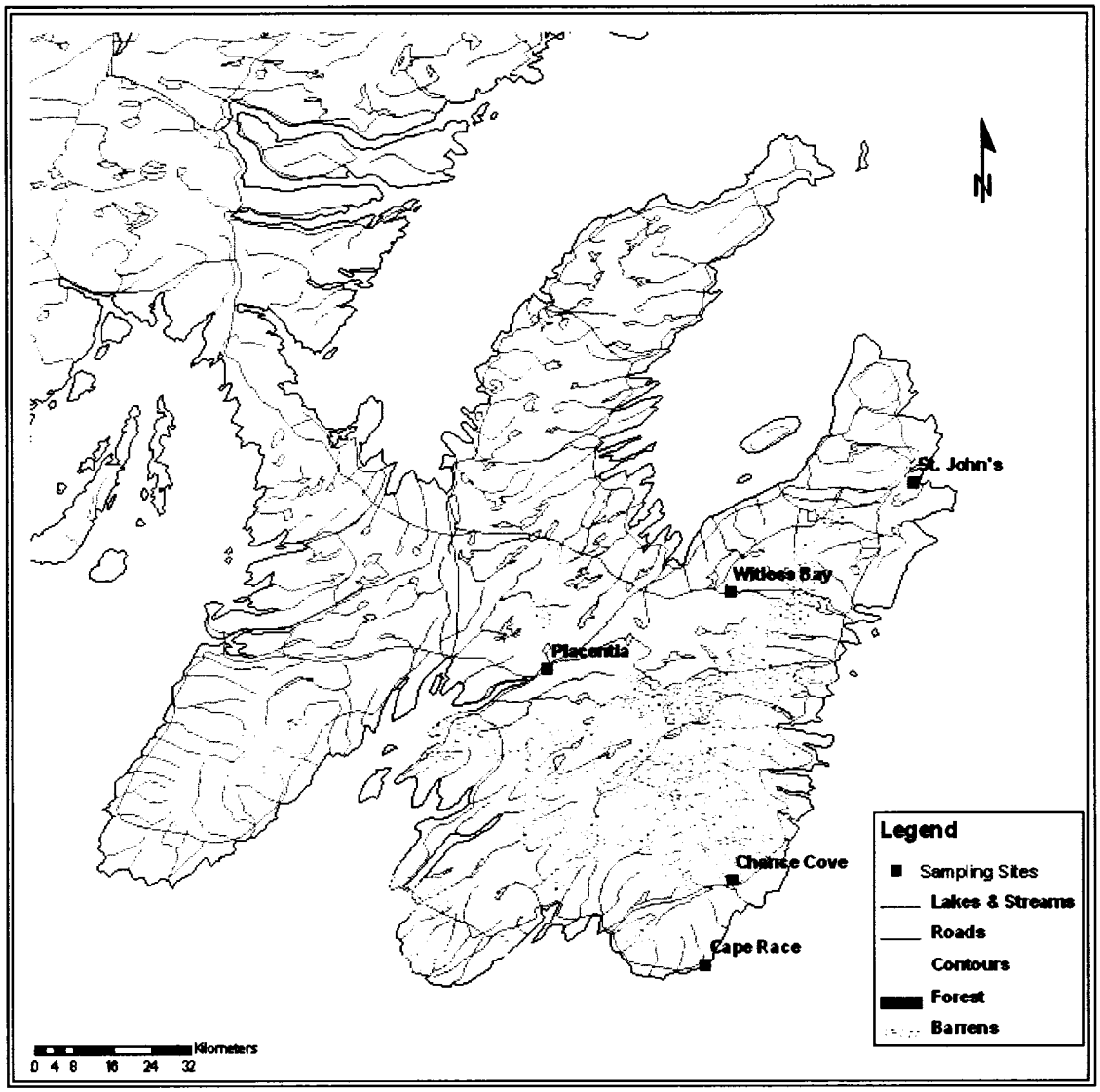

Figure 3.1 General areas of sites sampled.

Table 3.1 Number of sites sampled by landscape and location.

Site Categories		Sites Sampled
Barren	Outlet	6
	Downstream	10
	Total	16
Forested	Outlet	4
	Downstream	7
	Total	11
Total sites sampled		27

3.2.2 Seston Sampling Methodology

Seston chlorophyll-a was determined from a 2 L water sample collected at each site from fast flowing sections. Great care was taken to exclude particles disturbed from the substrate while collecting the water. Samples were kept on ice in a dark, thermal container and transferred to the lab where they were kept in the dark, on ice, until they could be filtered. Samples were filtered through Whatman glass microfibre filters (grade GF/C) with a 1.2 μm particle retention, using a vacuum pump attached to Millipore filtration equipment. To prevent the clogging of the filter, aliquots of 200-600 mL were taken depending on the particle concentration in the sample. A few mL of 1% magnesium carbonate solution was used to coat the filters to prevent chlorophyll a degradation. Filters were labeled and frozen until all samples were gathered. Then chlorophyll-a was extracted by placing the filters in glass tubes with 6mL of 90% acetone for 24 hours in a dark freezer. The level of chlorophyll-a and phaeophytin was measured using a fluorometer, and from these data the amount of chlorophyll-a and phaeophytin in mg/L was calculated (Eaton et al. 1995a).

Zooplankton was sampled at each site by pouring 50L of stream water at the sample site through a 100 μm mesh net, being very careful not to contaminate the sample with outside organic matter. The material retained by the net was transferred to a labeled, sealable glass jar and preserved in 70% ethanol. The number of zooplankton per sample was counted using a stereomicroscope.

3.2.3 Temperature Probes & Laboratory Experiments

Temperature, as a factor influencing larval distribution, was recorded at 20 sites from mid May to October 2003 using probes (VEMCO Ltd. MinilogTR). Eight were at

outlets with four in forested and four in barren landscapes. Eight were placed downstream from these outlets. Four additional probes were placed in streams that contained *P. apicalis* (as it did not occur at the above sites) to investigate the restriction of this species to cool streams.

Laboratory temperature tolerance experiments were conducted in the winter of 2002-2003 where larvae were kept at water temperatures of 15, 20, 25 and 30°C for two weeks to determine the effect of temperature on mortality.-Larvae were collected from rivers on the northeast Avalon Peninsula, transported to the lab on ice and transferred to 1L containers containing gravel, tap water, an air stone and a small amount of Tetramin fish food, which was ground into finer particles and then added. Food was added twice a week thereafter. Similar sized larvae were placed in each container as estimated by eye. Larvae were acclimatized for 24 hours at 15°C, and then temperatures were raised to the experimental levels. Water temperatures were checked at least every 24 hours and any dead larvae were removed and recorded. About 200mL of the water from each container was changed daily with water of the same temperature. After one week, the gravel was disturbed to determine the number of live larvae. Disturbance to those alive was kept to a minimum and they were left for another week.

3.2.4 Data Analysis

Data were entered into Microsoft Excel. Binary Logistic Regression was performed on the larval presence/absence data with Minitab version 14.1 to determine significant differences (α=0.05). Analysis of nutrient and larval abundance data, using only sites where they were present, was performed with SAS version 9.1. For count data

the Generalized Linear Model with a log link and a negative binomial distribution was used because of the non-normal distribution. If this distribution was not appropriate because of homogeneity of the residual deviance, then the Poisson distribution with a Pearson scale transformation was used. The chlorophyll-a data were a continuous response and thus the gamma distribution was used instead of the negative binomial. For the temperature data ANOVAs were carried out with Minitab version 14.1. Significant differences were determined at the α=0.05 level.

3.3 Results

Results from quantification of seston were highly variable. The chlorophyll-a content of the seston was significantly greater at outlets than downstream (p=0.0052) (Figure 3.2) as was zooplankton abundance (p<0.0001) (Figure 3.3). However, neither were significantly different by landscape nor were there significant interactions between location and landscape.

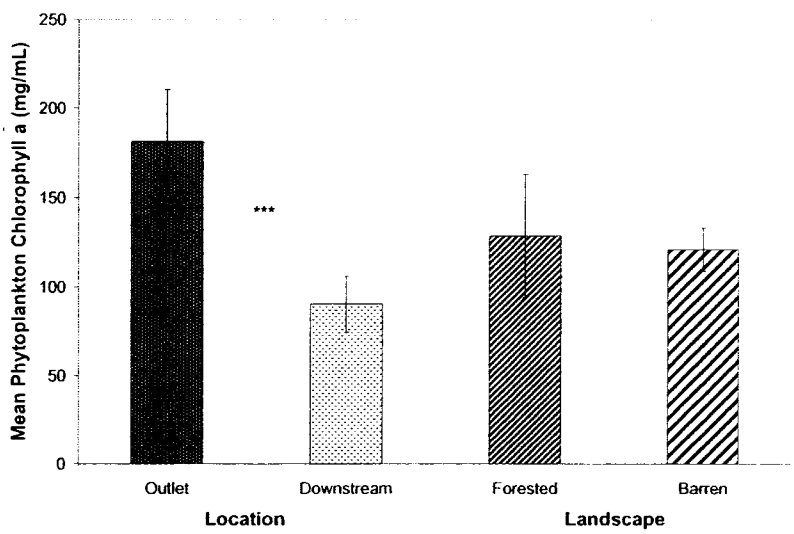

Figure 3.2 Mean chlorophyll-a concentration (mg/mL) by location and by landscape. *** denotes significance between groups of < 1%.

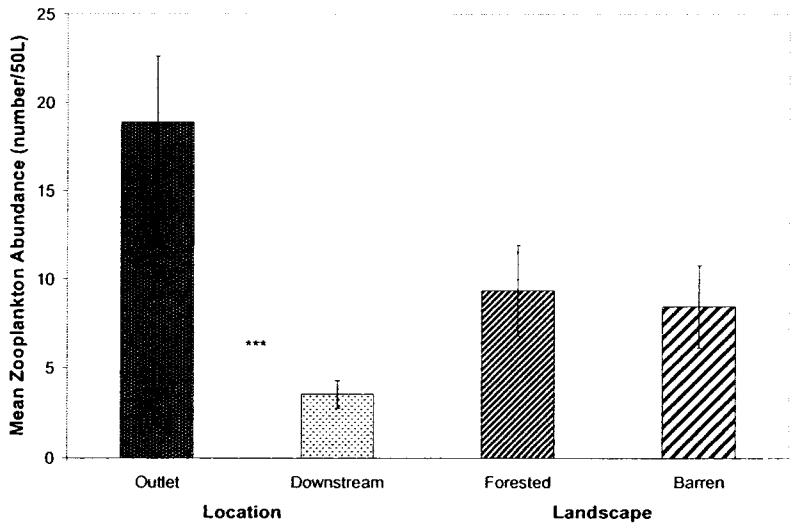

Figure 3.3 Mean zooplankton abundance (number/50L) by location and by landscape. *** denotes significance between groups < 1%. An outlier of 194 was omitted from outlet data.

Only *C. pettiti* and *H. betteni* were present at a significantly higher number of outlets and *H. sparna* was present at a greater number of downstream sites (Table 3.2). There were no significant differences in the presence of each species with landscape. A regression of the presence/absence data against plankton abundances, gave a positive relationship between phytoplankton and the presence of *C. pettiti* and *H. alternans.* With zooplankton a positive relationship was found with *C. pettiti* and *H. betteni* while a negative one was found with *H. sparna.*

Table 3.2 Frequency of occurrence of each species compared with location, landscape and plankton abundance. LO=lake outlet, DS=downstream, Frs=Forested, Brn=Barren

	Location			Landscape			Phytoplankton		Zooplankton	
Species	LO	DS	p	Frs	Brn	p	m	p	m	p
C. pettiti	9	6	0.004	8	7	ns	+	0.014	+	0.008
H. betteni	6	4	ns	5	5	ns		ns	+	0.022
H. sparna	6	15	0.028	9	12	ns		ns	-	0.008
H. slossonae	4	8	ns	5	7	ns		ns		ns
H. alternans	4	0	0.002	2	2	ns	+	0.002		ns
A. ladogensis	2	3	ns	1	4	ns		ns		ns
D. modesta	0	1	ns	1	1	ns		ns		ns
P. apicalis	0	4	0.043	1	3	ns		ns		ns
Immature	8	10	ns	8	10	ns		ns		ns
Total	10	16	ns	11	15	ns		ns		ns
n	10	17		16	11		27		27	

The abundance of each species was compared with location, landscape and plankton abundance (Table 3.3). Total abundance (all species) of larvae was significantly higher at outlets and in forested landscapes. The abundance of *C. pettiti* and *H. betteni* was significantly greater at outlets and no species had an increased abundance downstream. The abundance of *P. apicalis* was consistently low but was greater in forested landscapes, as was also true for immature specimens, and both had positive relationships with phytoplankton abundance. Abundances of *C. pettiti* and *H. betteni* declined significantly with increasing phytoplankton concentrations, but both species and *H. alternans* correlated positively to the abundance of zooplankton. Only *H. sparna* exhibited a significant negative relationship with zooplankton, having increased abundances downstream where the concentration of zooplankton declined.

Table 3.3 Comparing the abundance (mean and standard deviation (+/-)) of each species with location, landscape and seston abundance. Only sites where species were present were included in the analysis. The sign of the slope of the regression line is indicated by m. LO=lake outlet, DS=downstream, Frs=Forested, Brn=Barren

		Location			Landscape			Phytoplankton		Zooplankton	
Species	n	LO mean (+/-)	DS mean (+/-)	p	Frs mean (+/-)	Brn mean (+/-)	p	m	p	m	p
C. pettiti	26	349.31 (365.7)	3.86 (3.6)	<0.0001	223.6 (357.2)	262.5 (340.8)	ns	-	0.0319	+	0.008
H. betteni	19	182.27 (310.1)	24.23 (41.3)	0.0228	191.7 (325.2)	31.28 (43.7)	ns	-	0.0052	+	0.022
H. sparna	33	21.30 (29.0)	30.57 (41.0)	ns	30.29 (44.3)	26.68 (33.6)	ns		ns	-	0.008
H. slossonae	20	44.44 (82.6)	36.42 (40.5)	ns	32.92 (43.7)	43.66 (63.1)	ns		ns		ns
H. alternans	8	86.57 (65.9)	na	na	99.07 (65.8)	74.07 (73.3)	ns		ns	+	0.002
A. ladogensis	7	2.47 (1.7)	8.15 (12.2)	ns	1.23	7.41 (11.0)	ns		ns		ns
D. modesta	2	na	1.85 (0.9)	na	2.47	1.23	na		na		na
P. apicalis	6	na	22.22 (16.7)	na	30.25 (16.6)	18.21 (17.6)	0.0004	+	0.0003		ns
Immature	31	15.52 (13.8)	28.83 (46.9)	ns	29.63 (45.7)	16.44 (23.7)	0.0441	+	0.0213		ns
Total	51	482.2 (549.6)	69.89 (91.6)	<0.0001	294.5 (503.4)	183.9 (303.8)	0.0278		ns		ns

Stream sizes were categorized into five groups to facilitate analysis (Figure 3.4 & Figure 3.5). Phytoplankton abundance significantly differed among stream size categories ($p=0.0360$, Figure 3.4). Zooplankton abundance also differed among stream size categories, being much higher in the smallest streams ($p<0.0001$, Figure 3.5). The interaction of stream size and phytoplankton and zooplankton abundance was examined in relation to total hydropsychid abundance only, as small sample size did not allow consideration of individual species. Significant interactions were found between stream size, total hydropsychid abundance and both phytoplankton ($p=0.0101$) and zooplankton ($p=0.0284$). Phytoplankton abundance in the largest streams had a significant positive relationship with hydropsychid abundance ($p=0.0002$). Zooplankton abundance in the second largest stream width category (5 to 20m) had a significant positive relationship with hydropsychid abundance ($p=0.0002$).

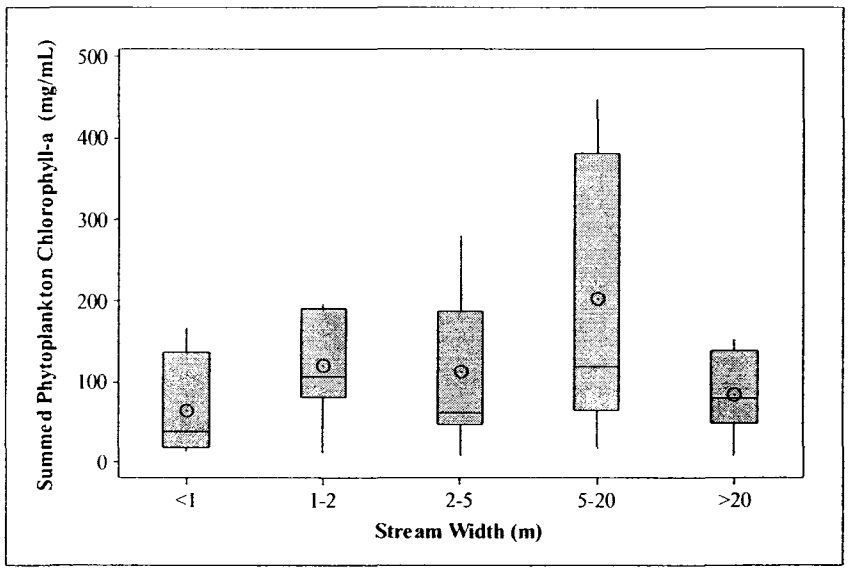

Figure 3.4 Summed phytoplankton abundance (chlorophyll-a (mg/mL)) versus stream width category. Dotted circles indicate the mean and horizontal lines indicate the median.

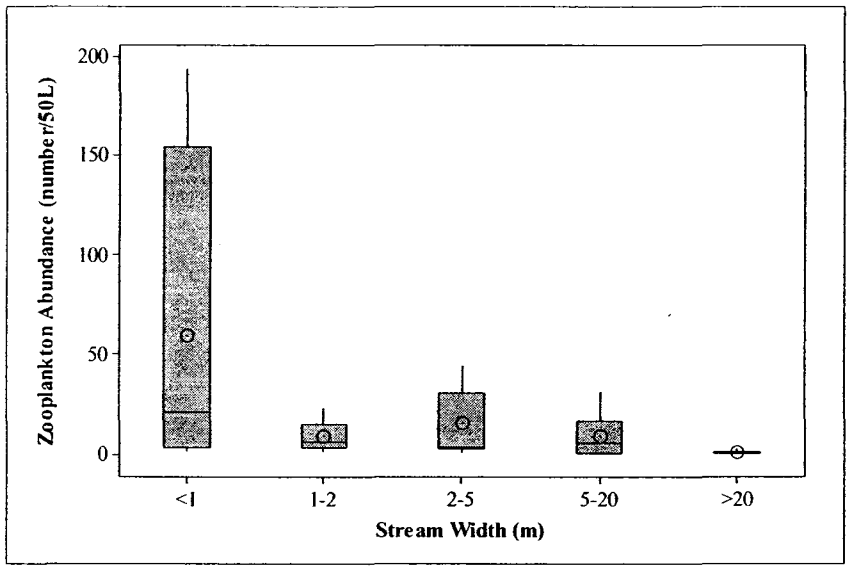

Figure 3.5 Zooplankton abundance (number per 50L) versus stream width category. Dotted circles indicate the mean and horizontal lines indicate the median.

Temperature comparisons among streams were limited to the first month of deployment (mid May to mid June 2003) as temperature probes had an upper limit of 20°C. Within the limits of this data set, outlets had a higher water temperature than downstream, forested rivers were warmer than barren rivers, and rivers thought to be spring or groundwater-fed, where *P. apicalis* occurred, were cooler than all other rivers measured. These differences were highly significant (α=0.05) (Table 3.4).

Table 3.4 Water temperatures, May 19 – June 19 2003, compared with location, landscape and (presumably) groundwater-fed streams, where vs.=versus.

	Temperature (°C)		
Habitat	mean	+/-	p-value
Outlet	11.90	2.3	<0.0001
Downstream	11.67	2.6	
Forested	12.42	1.9	<0.0001
Barren	11.12	2.8	
Groundwater-fed	9.86	2.6	<0.0001 vs. downstream <0.0001 vs. barren

Results from the laboratory experiment of the effect of temperature on species survival (Figure 3.6) show a significantly lower level of survival for *P. apicalis* at temperatures of 25°C and 30°C compared to the other species tested (p=0.034). The other species had high frequencies of occurrence (Chapter 2) and this experiment indicates that they were able to tolerate a wide range of temperatures.

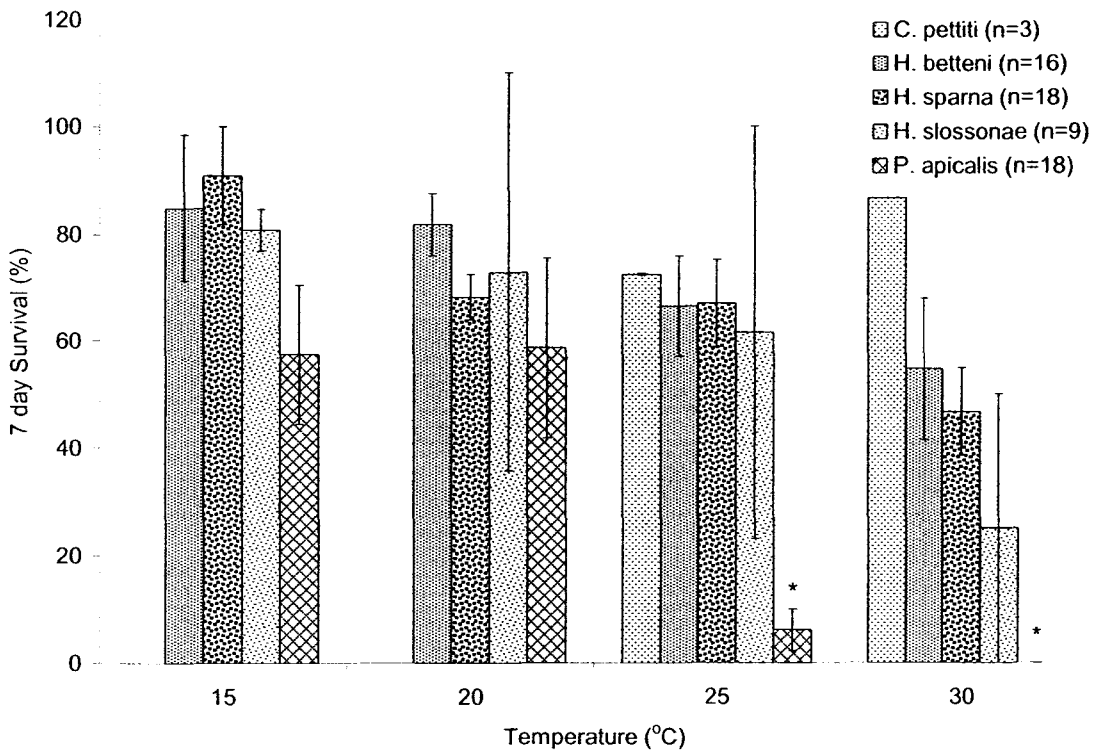

Figure 3.6 Effect of temperature on the survival of five species of hydropsychids in seven day laboratory trials.

3.4 Discussion

3.4.1 Effect of location

Lake outlets had significantly higher abundances of phytoplankton and zooplankton in comparison to downstream. This increased concentration out-flowing from lentic bodies is well documented (Naiman 1983; Oswood 1979; Woodward &

Hildrew 2002; Yusoff et al. 2002). The overall significantly greater abundance of hydropsychids at outlets compared to downstream sites was a pattern commonly found elsewhere (Parker & Voshell 1983; Ross & Wallace 1983; Spence & Hynes 1971; Valett & Stanford 1987). Several other factors contribute to this increased abundance including higher water temperatures, laminar flow, the relatively constant presence of flowing water even at low water levels and low levels of sediment because lakes act as sediment sinks. Lack of sediment reduces scouring of the stream bottom which is of great benefit to fixed retreat builders. There is an abundance of food resources and the habitat is generally stable and thus larvae are able to achieve high densities by co-existing in close proximity to each other (Richardson & MacKay 1991).

Three species, *H. alternans, C. pettiti* and *H. betteni* showed an increased presence and abundance at outlets and exhibited a positive relationship with zooplankton. *Cheumatopsyche pettiti* is known to favour warm, enriched waters (Kondratieff et al. 1997) and was rarely found downstream in this study. *Hydropsyche althernans* and *H. betteni* are reported to be carnivorous, so it is logical for it to have increased densities at outlets where zooplankton levels are high (Fuller & MacKay 1980a; Milne 1943).

Hydropsyche sparna had a higher occurrence and abundance downstream, which negatively correlated with zooplankton abundance. It is a generalist feeder and may obtain sufficient nutrition from sources other than zooplankton, thereby avoiding competition with dense filter feeding populations at outlets (Fuller & Mackay 1981). This distribution is similar to that found by Morin & Harper (1986) in Quebec where *H. sparna* was found downstream from *C. pettiti* and *H. betteni,* species which were dense at

the outlet. The widespread distribution of *H. sparna* among streams may reflect a more efficient utilization of lower quality downstream seston compared to other hydropsychids (Fuller & Mackay 1981). Large quantities of seston flowing out of lakes rapidly decline with increasing distances from outlets (Vadeboncoeur 1994). In streams without lentic bodies the River Continuum Concept would predict allochthonous material can be a major source of energy that influences downstream communities more so than outlets because this material is being reduced to finer particulate matter by stream biota (Benke 1984) and is being carried downstream by the flow (Vannote et al. 1980). However, allochthonous material may be of lower quality because of the greater energy costs required by an organism to utilize it as a food source (Fuller & MacKay 1980a) thus densities of hydropsychids would not generally reach those seen at outlets (Benke 1984; Haefner & Wallace 1981).

In the current study, the occurrence and density of the remaining species did not differ by location. The low densities of *H. alternans, D. modesta* and *P. apicalis* made it not possible to compare their abundances with all factors. More extensive research needs to be conducted on rivers where these species have higher densities in order to more fully understand the factors governing their distribution.

3.4.2 Effect of landscape

The occurrence of each species did not significantly differ between forested and barren landscapes; they had an equal probability of occurring in either landscape. However, the overall density of total hydropsychids was significantly higher in forested landscapes, which may be because of differences in food quantity and/or composition

with landscape. Forested landscapes may contribute higher quantities and/or a higher quality of allochthonous material than barren landscapes because of the nature of the surrounding vegetative patterns (Hauer & Lamberti 1996). Allochthonous material can be a substantial nutrient source and thus the elevated nutrient potential in forested landscapes may contribute to the increased overall abundance of hydropsychids (Vannote et al. 1980).

The density of *P. apicalis* was consistently low, but was slightly higher in forested landscapes than in barren landscapes. More extensive and equal sampling in cooler rivers in both landscape types would provide a stronger basis for analyzing the influence of landscape on this species. *P. apicalis* utilizes woody debris and mosses (Flint 1961; personal observation) to construct retreats and the presence of this material may affect its distribution, although this is not likely the case here.

The quantity of phytoplankton and zooplankton significantly differed with location but not with landscape type in this study; however the quality may have differed with location and landscape because of selective ingestion of higher quality foods by hydropsychids. Laboratory studies of hydropsychid feeding selectivity show increased uptake of plankton (Fuller et al. 1983; Fuller et al. 1988; Fuller & Fry 1991; Fuller & Mackay 1981; Fuller & MacKay 1980b; Fuller & MacKay 1980a; Petersen 1985; Petersen 1987b; Petersen 1987a; Petersen 1987c). The species composition of the plankton was not considered here, but may have changed with landscape and location and contributed to a shift in the hydropsychid community composition. A given species may select certain types of plankton and thus its occurrence and density may be correlated

with the composition of the plankton. More extensive research is needed on the species composition and nutritive quality of the seston.

Food which is easier to digest, such as animal food rich in protein or some algae, may be a preferred food choice. Certain species of algae have been shown to be selectively removed from the plankton (Valett & Stanford 1987). Algae may not always be digested because viable unbroken algal cells may pass through hydropsychid guts (Benke & Wallace 1980). The palatability of algal cells is also known to differ, which may lead to selectivity in uptake and digestion (Wallace & Merritt 1980). Therefore the species composition of the seston is important when considering seston quality. Previous gut content analyses have shown differences in feeding preferences by hydropsychid species (Morin & Harper 1986; Petersen 1985; Wallace 1975a). Genge (1985) showed feeding differences among hydropsychids in a Newfoundland stream, with *H. betteni* being the most carnivorous, followed by *H. slossonae, H. sparna* and finally *C. pettiti.* This type of analysis was beyond the logistic capabilities of the current study.

The plankton concentration from a lentic outflow is highly variable and is influenced by climatic factors. Large rainfalls cause spates which flush the lake and can quickly carry plankton downstream (Campbell 2002). Excessively warm temperatures and/or lack of wind prevent a lake from mixing which inhibits plankton production and reduces oxygen content of the out-flowing water (Bronmark & Hansson 1998). Most lakes above outlets sampled here were likely well mixed as the lakes were shallow and subject to frequent winds. All samples in this study were taken in the early spring and stratification would have not yet occurred. Nevertheless a higher frequency of sampling

would have provided a better measure of plankton composition and abundance over time, which could have been related to the feeding dynamics and densities of the hydropsychids.

3.4.3 Effect of temperature

Lake outlets were significantly warmer than downstream sites, a phenomenon commonly found elsewhere (Richardson & MacKay 1991; Wotton 1995). Water temperatures in forested sites were significantly higher than barren sites, a comparison not known to have been previously investigated. Temperature can influence lotic communities (Giller & Malmqvist 1998; Hynes 1970a) particularly the hydropsychids (Edington & Hildrew 1973; Hildrew & Edington 1979). Higher temperatures cause increased feeding and digestion rates, increased metabolic and respiration rates, and higher growth rates of aquatic insects (section 1.5.1) (Freeman & Wallace 1984; Giller & Malmqvist 1998; Wallace & Merritt 1980). Increased growth rates lead to faster progression through larval instars and so shorter periods of time are needed to complete life cycles (Giller & Malmqvist 1998). Here, at a small, warm lake outlet it was observed that the larval size of *C. pettiti* and *H. betteni* was variable and sizes of pupae were smaller compared to those in nearby streams, which could reflect food availability and temperature as observed in Simuliidae occurring at outlets (Colbo 1982; Colbo & Porter 1979).

Arctopsyche ladogensis found in cooler sites here agrees with previous studies (Flint 1961; Hauer et al. 1989). *Diplectrona modesta* was rare, but nearly always found at cool, downstream sites in smaller streams. *Hydropsyche slossonae* was widely distributed

here (occurring at outlets and downstream sites and in mid to larger streams) but has been reported to occur in large, cool rivers (Schuster & Etinier 1978) suggesting temperature may not be influencing its distribution in Newfoundland(Schefter & Wiggins 1986). However, in laboratory trials both *H. slossonae* and *H. sparna* showed marked declines in survival at the highest water temperatures (Figure 3.6).

Parapsyche apicalis occurred in cool streams here, which agrees with its tolerance elsewhere as reported by Flint (1961). This suggests that lower water temperature tolerance may be limiting its distribution in Newfoundland to cooler streams, presumably groundwater-fed. The laboratory experiments reinforced this intolerance to high temperatures. The Oxen Pond outlet seemed contradictory being an outlet, but the water temperature probe data showed this site to be cool. This area is known to have several springs and it appears there was an influx of groundwater between the outlet and where *P. apicalis* was found.

3.5 Conclusion

In conclusion, the distribution of Hydropsychidae in Newfoundland was greatly influenced by the presence of outlets, a habitat shown to have high concentrations of nutrients and warmer temperatures in this study. Densities of hydropsychids were elevated at outlets and were greater in streams surrounded by forested landscapes. Detailed investigation is required to determine the factors which cause changes in the occurrence/density of the hydropsychid community with respect to distances from outlets and the influences of landscape. For example, do changes in the hydropsychid community reflect those of composition and/or quality of the seston? If so, how does this impact larval feeding behaviour? Are these relationships influenced by landscape?

4. CHAPTER 4: DEVELOPMENT OF A COMPARATIVE MODEL TO EXAMINE THE INFLUENCE OF LAKE OUTLETS ON THE DISTRIBUTION AND ABUNDANCE OF HYDROPSYCHIDAE IN STREAMS OF DIFFERENT SIZES ACROSS FORESTED AND BARREN LANDSCAPES

4.1 Introduction

In stream systems lentic bodies are both a sink and a source for the materials moving downstream, interrupting processes modeled by the River Continuum Concept of Vannote et al. (1980) and influencing the structure and abundance of the lotic community immediately downstream of outlets (Richardson & MacKay 1991). For example, lake waters have low sediment concentrations, altered nutrient levels, higher temperatures and contain phytoplankton and zooplankton; all of these effects decrease with increasing distance downstream from outlets (Chapter 3) (Chandler 1937; Hildrew & Edington 1979; Maciolek & Tunzi 1968; Newbold et al. 1982). This chapter investigates hydropsychid distribution below lake outlets and that of their potential nutrients (phytoplankton and zooplankton) emanating from lakes to determine if hydropsychids are following a predictable pattern away from outlets and if this is a reflection of nutrient abundance. Periphyton abundance is also measured, mainly as an indicator of stream productivity. In order to sample multiple streams of varying sizes a model was devised, based on small particles emanating from outlets, for the establishment of sampling points that were comparatively equidistant from outlets.

Previous work on Hydropsychidae in Newfoundland (Chapters 2 & 3) has shown that outlet communities are dominated by two species, *Cheumatopsyche pettiti* and *Hydropsyche betteni,* which were rarely found downstream where *H. slossonae* and *H.*

sparna are dominant taxa. From this arises the question of whether the hydropsychid community changes with increasing distance from outlets in a definable pattern. Previous work (Chapters 2 & 3) determined there were differences in the presence and abundance of hydropsychids by location (outlets versus downstream sites) and by landscape (forested versus barren landscapes). Therefore could these changes be modeled to predict hydropsychid abundance at a given point in a stream? For example, how quickly do the species commonly found at outlets, *C. pettiti* and *H. betteni,* decrease in abundance with increasing distance downstream and what is the influence of landscape? Is there a consistent pattern amongst streams of different sizes?

Understanding the factors contributing to the distribution and abundance of hydropsychids is important as they are integral to trophic functioning in stream ecosystems (Oswood 1979; Wiggins 1996). Hydropsychids are filter feeders, capturing seston in the stream which emanates from lakes. Seston composition influences hydropsychid biomass and abundance (Ross & Wallace 1982), and so understanding lake influences is an important aspect of hydropsychid ecology (Petersen 1987c; Ross & Wallace 1983).

Lakes are abundant in Newfoundland's glaciated stream systems and are generally oligotrophic, so seston concentrations may rapidly decline compared to seston from more enriched lentic bodies elsewhere where an increased seston concentration would be carried further downstream. How does the quantity of seston change with increasing distance from lake outlets in Newfoundland streams? Is the rate of change in

seston quantity correlated with the change in the hydropsychid community in terms of species abundance?

Sheldon & Oswood (1977) developed a mathematical model to predict the dependence of filter feeder abundance on the amount of seston emanating from outlets. It is a negative power function of the equation $N_D=aD^{-b}$, where N_D is the number of filter feeders, a is the intercept, D is the distance downstream from the outlet and b is the slope. It was initially tested using blackfly larvae in Owl Creek Montana (Sheldon & Oswood 1977) where abundances generally agreed with the model. Then hydropsychid abundances were tested in the same stream (Oswood 1979) and found to generally agree with the model. Vadeboncoeur (1994) found components of the seston in Owl Creek (particulate organic carbon (POC), bacteria and chlorophyll-a) sometimes fit a power function, and that the slope was related to discharge. Eriksson (2001) tested the model on four Swedish streams but found no relationship between filter feeding caddisfly abundance and zooplankton abundance.

A negative power function was therefore a useful test model for exploring the rate of change of the hydropsychid population below lakes in Newfoundland streams. To develop a basis for comparison among Newfoundland streams a model was required which predicted the distance of downstream transport of particles originating from lakes in streams of different sizes so that sample points were located at analogous distances on multiple streams. This was necessary because the rate of decrease of seston in a stream is dependent on available energy to maintain the material in suspension, which is dependent on discharge (Morisawa 1985). Discharge is correlated with stream width, depth and

velocity (Newbury 1984). Discharge varies with stream size and so influences downstream transport of lake plankton (Walks & Cyr 2004).

Downstream particle movement follows an inverse logarithmic scale (Morisawa 1985), with many particles traveling only short distances from the source. The number of particles transported further downstream rapidly decreases as distance from the outlet increases (Chandler 1937). It was necessary to determine the rate of this decrease to optimally plan a sampling program to address the questions posed in this study. Previous work on the development of *Bacillus thuringiensis* var *israelensis* (BTI) for the control of blackfly populations had been conducted in the Province, and the effective distances of downstream transport of the bacterial pesticide from a dose point was determined (Colbo 1984; Lacey & Undeen 1984). The particulate BTI was assumed to have a similar transport to that of seston and thus could be used to develop a model for the rate of decrease of seston particles emanating from outlets. This rate was determined iteratively, and it was found that particle abundance decreased at a rate of 1.8 on a log scale. This value was tested against previous studies on the Island (Colbo 1984) and found to concur with previous results. The equation for the rate of change of particle abundance with increasing distance from a lake outlet was:

$$\mathbf{y\ (x) = e^{i} * e^{-0.5878x}}$$ (Equation 4.1)

where **y** is the particle abundance at a given distance **(x)**; $\mathbf{e^{i}}$ is the initial particle abundance at the outlet (the intercept); **-0.5878** is the slope of the regression, the rate of change in particle abundance with distance downstream; and **x** is the distance

downstream. Note that the ln (1/1.8) = -0.58778664902119, where the inverse is taken because of it being a decay (decrease) function and the number was rounded to four decimal places for ease of use.

The rate of decrease of particle abundance is partly a function of discharge, which is correlated with stream width at a riffle (Colbo 1984). However width as a surrogate measure for stream discharge is not linear. For example, in smaller streams particles will fall out faster because of the decreased inertia of the smaller volume of water related to the larger wetted surface to volume ratio which increases the influence of drag (Morisawa 1985). Thus width of a stream must be incorporated into the above model. Stream width was measured at outlets as this was the point source of the particles and therefore the intercept or the starting point of the regression of interest. Thus the above equation became:

$$\mathbf{y\ (x) = width * e^{i} * e^{-0.5878x}}$$ (Equation 4.2)

In order to compare streams it was necessary to determine sampling points that were at analogous distances downstream from outlets. To determine relative sampling points in streams of different sizes equation 4.2 needed to be modified by removing the particle concentration parameter. The interest was not in modeling particle abundance but in determining relative sampling points while incorporating changes in stream widths as a surrogate for discharge. This provides analogous distances at which to sample streams, so equation 4.2 became:

$y(x) = \text{width} * e^{-0.5878x}$ (Equation 4.3)

where **y** is the distance downstream from the lake outlet in metres and **x** is a positive integer. Table 4.1 gives values of y for streams of different widths using equation 4.3. Values of x of zero (the outlet), two, four, six and eight were chosen in order to sample frequently near the lake outlet as this was where the greatest rate of change was postulated to occur (Richardson & MacKay 1991), and to cover a sufficient distance downstream to reach beyond the limits of seston transport determined in Chapter 3. These sampling points are hereafter referred to as stations.

If this model of decrease, essentially a decay model, was correct, each of the five stations in any size of stream would be relatively the same distance from the lake outlet with respect to seston entering the stream from the lake. This permitted testing of factors that may influence the patterns seen in Chapters 2 & 3 by allowing direct comparisons of the structure and abundance of hydropsychid communities among streams of different sizes as well as between streams in barren and forested landscapes at analogous distances from a lake.

A statistical method was used to test the derived model to the collected data, using a regression with a model equation of:

$y(x) = e^{i} * e^{\beta x}$ (Equation 4.4)

where **y** is the abundance of seston or hydropsychids; e^{i} is the abundance of seston/hydropsychids at the outlet, the intercept; β is the slope of the decay function; and **x** is the distance downstream. The statistical regression method estimates parameters for i

and β, and the 95% confidence limits of β should contain the derived model slope (-0.5878) if the data are to fit the derived model.

Table 4.1 Computation of stations (distances downstream at which to sample) measured in metres from a lake outlet. Values are determined by equation 4.3 for values of x from zero to 10, and for streams of arbitrary width for exemplary purposes. Grey areas depict stations used in this study.

		width (m)								
Station	x	1	2	4	8	10	15	20	35	40
0	0	0	0	0	0	0	0	0	0	0
	1	1.80	3.60	7.20	14.40	18.00	27.00	36.00	63.00	72.00
2	2	3.24	6.48	12.96	25.92	32.40	48.60	64.80	113.40	129.60
	3	5.83	11.66	23.33	46.66	58.32	87.48	116.64	204.12	233.28
4	4	10.50	21.00	41.99	83.98	104.98	157.46	209.95	367.42	419.90
	5	18.90	37.79	75.58	151.17	188.96	283.44	377.91	661.35	755.83
6	6	34.01	68.02	136.05	272.10	340.12	510.18	680.24	1190.43	1360.49
	7	61.22	122.44	244.89	489.78	612.22	918.33	1224.44	2142.77	2448.88
8	8	110.20	220.40	440.80	881.60	1102.00	1652.99	2203.99	3856.99	4407.98
	9	198.36	396.72	793.44	1586.87	1983.59	2975.39	3967.19	6942.58	7934.37
	10	357.05	714.09	1428.19	2856.37	3570.47	5355.70	7140.93	12496.64	14281.87

4.2 Materials and Methods

4.2.1 Study Area

Eight streams were selected on the Avalon Peninsula, four from landscapes with predominately forested vegetation cover (hereafter called forested), and four from sites dominated by low growing ericaceous shrubs, stunted clumps of conifers and lichens and mosses (hereafter called barren) (Damman 1983) (Appendix 2 (section 10.2)). Landscape characteristics were described in greater detail in Table 1.5. Streams were chosen in four size ranges based on the width at outlets, giving a gradient from small to large streams (Table 4.1). Streams with similar width ranges were selected in both landscape types. The locations of the streams sampled are shown in Figure 4.1. The distance of stations from the outlet were determined using equation 4.3 where stream width was measured at the first riffle below outlets. Table 4.2 gives the distance of each station from the outlet for

the eight streams. As hydropsychids inhabit faster moving waters, rock bag samplers were placed in riffle habitats closest to the calculated distance for stations.

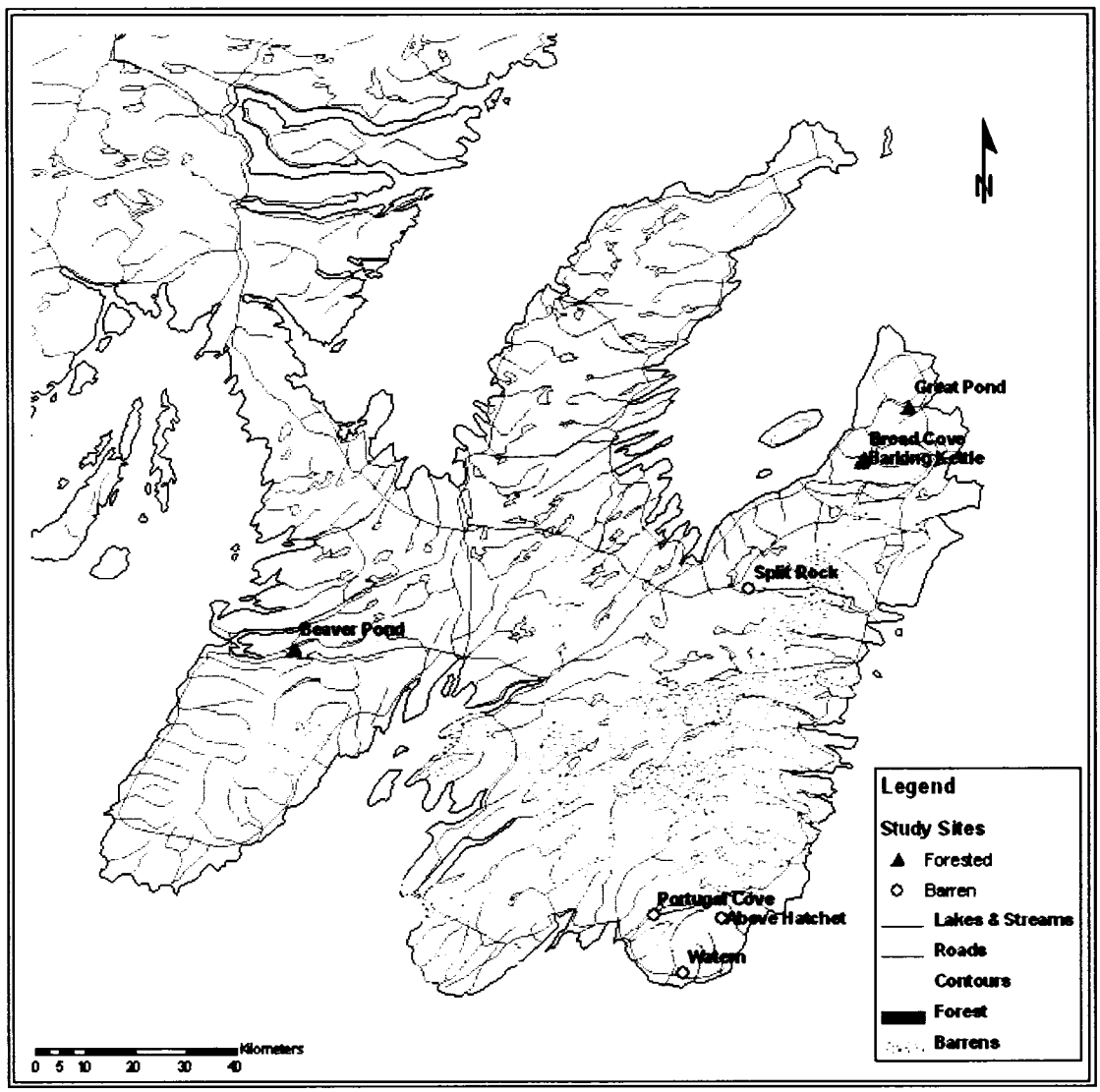

Figure 4.1 Map of the locations of the study sites on the Avalon Peninsula of Newfoundland, Canada.

Table 4.2 Classification of the streams sampled. Distances are relative to outlets (measured from outlets) in metres.

Stream	Barking Kettle	Great Pond	Broad Cove	Beaver Pond	Split Rock	Above Hatchet	Watern	Portugal Cove
Abbreviation	BK	GP	BC	BP	SR	AH	WT	PC
Landscape	Forested	Forested	Forested	Forested	Barren	Barren	Barren	Barren
Width at Outlet (m)	1	1.5	3.5	14	1.5	1.75	2.5	23
Station 0 (m)	0	0	0	0	0	0	0	0
Station 2 (m)	3	4.86	11.5	51	4.3	6	8.1	75
Station 4 (m)	7.5	5.75	36.5	208	16	32	34	241
Station 6 (m)	26.5	59	119	~476	51	60	85	~800
Station 8 (m)	110	158	388	~1543	165	193	275	~2400

4.2.2 Sampling Hydropsychidae

Rock bag samplers were used to collect Hydropsychidae during this study. These were created by cutting approximately 30cm sections of expandable, cylindrical, plastic mesh netting (Vexar® www.masternetltd.com) with diamond shaped openings of 2cm by 2cm and tying one end closed with twine. Into this formed 'bag' was placed approximately 1.5L of rocks, about 4-6cm in diameter, and then the other end was closed with twine. Three rock bag samplers were placed in riffles in close proximity to each other at each of the five sampling stations along each stream. They were placed in wadeable riffles in rapid flow, where they were wedged between larger substrates to prevent downstream movement during normal spates.

Rock bag samplers were placed in streams in mid May of 2003 and left to colonize for five weeks. The samplers were recovered by placing a 250μm mesh sieve downstream of the bag and lifting it into the sieve which was then removed from the stream. The bag was then placed in a bucket of water where it was agitated vigorously. The water from the bucket was poured through the 250μm mesh sieve and the process was repeated until no more organisms could be recovered from the bag. The material collected was transferred to a labeled freezer bag and preserved by adding sufficient 95% ethanol to the estimated sample volume to have a minimal concentration of approximately 70% ethanol in the sample. At the first, second and third sampling times rock bags were placed back in the riffles to be colonized for three weeks, permitting four collections: mid June, early July, late July to early August, and late August.

In the laboratory all hydropsychid larvae and pupae were removed from the samples with the aid of a dissecting scope. They were identified to species using keys (Rutherford 1985; Schefter & Wiggins 1986; Schuster & Etinier 1978), enumerated and stored in labeled glass vials in 70% ethanol.

Rocks used in the rock bags were not uniform in size as bags at remote locations needed to be filled on site. Although the volume and size range of the rocks used was kept as constant as possible, there was variation amongst the rock bags. The total surface area represented by the rocks in an individual bag could have differed, altering potential area for hydropsychid colonization. To correct for this, three rock bags were randomly chosen from each stream and the maximum length and width (rock area) of 10 randomly selected rocks in a bag were measured. To obtain an estimate of the total rock surface area per bag, random samples were taken from these measurements to obtain a mean rock surface area given the number of rocks in the bag, and the total rock surface area for that bag was calculated. This was done for each of the three rock bags randomly removed from the 15 rock bags used in each stream. The average of these three was the estimated rock surface area for the bags in a given stream, calculated for each of the eight streams. Differences in rock surface area among streams were then standardized. This was done by calculating the ratio of the rock surface area in a given stream to that of the stream with the largest rock surface area and the hydropsychid abundance was adjusted accordingly.

4.2.3 Sampling physiochemistry, plankton and periphyton

At each station, pH, conductivity and water temperature were measured with a YSI 85 meter. Two water velocity readings were taken with an Ott meter on the upstream edge of each rock bag sampler. Phytoplankton was sampled at each station by collecting duplicate 1 L water samples in plastic bottles, carefully avoiding surface films and bottom particles. These were labeled and kept on ice in the dark for transport. In the lab the water was vacuum filtered with a Millipore filtration system through Whatman GF/C glass fibre filters with a 1.2 μm pore size. A few mL of 1% magnesium carbonate were added to coat the filter to prevent phytoplankton degradation. Filters were folded in half, labeled and kept frozen at -20°C until extraction.

Phytoplankton quantities were obtained by measuring chlorophyll-a and phaeophytin using a flurometer. Filters were placed in glass tubes with 6 mL of 90% acetone for 24 hours in a dark freezer and then placed in the flurometer. The acetone extract was placed in a flurometer and from these readings the amount of chlorophyll-a and phaeophytin in mg/L was calculated as outlined by Eaton et al. (1995a).

Zooplankton was sampled by pouring 50 L of stream water at each station through a 100 μm mesh sieve. Care was taken not to disturb the bottom sediments. The sieved material was washed into a labeled, sealable glass jar using 70% ethanol as a preservative. In the lab, under a stereomicroscope, the zooplankton were enumerated and identified to either family or genus using the characters that could be distinguished at 50x magnification.

Periphyton (plants attached to benthic substrates (Weitzel 1979)) was sampled by placing unglazed ceramic tiles on the stream bottom at each station. Three 7.5 cm^2

ceramic tiles were glued one cm apart onto a cloth strip with silicone. Tiles were placed smooth side up in a riffle at each station and secured by placing rocks over the ends of the cloth strip. After three weeks colonization, the tiles were removed, carefully cut apart so as not to scrape the surface and placed in labeled, sealable plastic bags. They were kept on ice in the dark for transport to the lab where they were frozen at -20°C until extraction. Chlorophyll-a was extracted from the surface of the tiles using 90% acetone for 24 hours in a dark freezer. Levels of chlorophyll-a and phaeophytin were measured in a flurometer. The amount of chlorophyll-a (mg/m^2) per tile was calculated as outlined Eaton et al. (1995a).

Conductivity, pH and velocity were measured during all four sampling times. Plankton, periphyton and zooplankton were collected during the last three sampling times, from July to August 2003, as tiles were placed in the stream during the first sampling time in June.

4.2.4 Data analysis

The goal was to model the change in the abundance (number per rock bag) of hydropsychids and seston with increasing distance from outlets, which was the slope of the regression curve between abundance and distance downstream. Regressions were conducted using the Generalized Linear Model (GLzM) with a log link and a negative binomial distribution (for counts of hydropsychids and zooplankton) or a gamma distribution (for continuous responses such as periphyton and phytoplankton) in SAS version 9.1. These distributions account for the non-normal error structure of the data caused by the nature of hydropsychid abundances, with some counts of very high

abundances but also many zero counts as not all species were found at each station along the longitudinal gradient. Here, longitudinal refers to the change in abundances from outlets to downstream stations. The null model in this case was that there was no change in abundance with increasing longitudinal distance with significant differences determined at the α=0.05 level. Of greater interest was the rate of change of abundance, or the slope of the line which is a parameter estimated using the GLzM, including its 95% confidence limits. The GLzM also estimates a second parameter, the intercept, which in this case is the hydropsychid or seston abundance at the outlet. The comparison of these parameter estimates to the derived model determines how well the derived model fits the data. If the slopes were not equal, but the derived model fit within the 95% confidence limits then the derived model was regarded as a reasonable fit to the observed data.

Chlorophyll-a concentrations of the periphyton and phytoplankton (hereafter referred to as abundances of periphyton and phytoplankton) as well as zooplankton abundances (number/50L) were directly compared to hydropsychid abundances using logistic regression. Also of interest was the overall longitudinal distribution of the abundance of periphyton, phytoplankton and zooplankton and whether they followed a similar trend to that of the hydropsychids, which would indicate a potential relationship with hydropsychid abundance. If phytoplankton and zooplankton were emanating from outlets then their abundance was expected to decrease with increasing distance from outlets. A significant longitudinal change in the periphyton abundance was not expected because stream productivity was thought to be independent of outlet influences. The rate of decrease of periphyton, phytoplankton and zooplankton abundances was compared to

the rate of decrease of hydropsychid abundances to determine if they followed similar trends. This was done using an ANCOVA with a negative binomial distribution to account for the non-normal error structure. A log link was used for phytoplankton and zooplankton and an identity link was used for periphyton. If there was not a significant interaction between the slopes of hydropsychids and phytoplankton/zooplankton/ periphyton then their slopes would not be significantly different and hence they would be following a similar trend.

The derived model predicted that hydropsychid abundance would decline from the lake outlet at a rate of $e^{-0.5878}$. Did this model fit the data, especially for the two species with the greatest abundances at outlets? To determine this, the slopes of the regression lines, regressing abundance with distance downstream, were compared to determine if they were significantly different. Of greater interest were the parameter estimates of the slope, to determine if the confidence limits estimated by the collected data enclosed the derived model. In order to model a species' abundance, a minimum of 200 individuals of a species recovered in total from a given stream was set. This criterion was based on recovering a mean of ten individuals from each station for the four sampling times.

A canonical correlation was used to compare the physical/chemical variables with hydropsychid abundances. This procedure standardized the data by subtracting the mean and dividing by the standard deviation; this makes the mean zero, the variance and standard deviation one, and values are dimensionless. This allows for comparison amongst variables of different units on the same scale. In this analysis, the first two axes

of a PCA among the physical/chemical/nutrient factors were compared to the first two axes of a PCA among the species abundances.

4.3 Results

4.3.1 Hydropsychidae abundance with longitudinal distance

Hydropsychid species' abundance varied greatly between the eight streams when considering the combined total of the four sampling times (Table 4.3). The most abundant species at outlets were *Cheumatopsyche pettiti* and *Hydropsyche betteni* (Figure 4.2 & Figure 4.3), whose abundances sharply declined downstream where hydropsychid communities were dominated by *H. sparna* and *H. slossonae* (Figure 4.2 & Figure 4.3). Three species (*H. alternans, Arctopsyche ladogensis* and *Diplectrona modesta*) did not occur in all streams and their abundances were low. *Hydropsyche alternans* was closely associated with outlets but found in only two streams (Figure 4.2 & Figure 4.3; Table 4.3). *Arctopsyche ladogensis* and *D. modesta* were found in only three streams and only at the most distant downstream sites in low abundances (Figure 4.2 & Figure 4.3; Table 4.3). *Parapsyche apicalis*, a species found throughout the region in other streams (Chapter 2), was not found in these eight streams. Small larval hydropsychids which could not be identified to species, grouped here as immature, were encountered in high abundances in all streams, but their longitudinal abundance differed with time (Figure 4.2 & Figure 4.3; Table 4.3). Very few immature larvae were recovered in June, but in July outlet samples consisted of ~60% immature larvae, with the proportion of immature larvae increasing in the downstream samples during the third and fourth sampling regime. There was much variation over time in the proportion of each species at each station.

However, the general trends of *C. pettiti* and *H. betteni* dominating outlets and *H. sparna* and *H. slossonae* having greater abundances downstream held throughout this study. It is interesting to note that as the abundance of *C. pettiti* declines to low levels at station four, the abundance of *H. sparna* increases from station four onward (Figure 4.2).

Figure 4.3 shows the mean abundance of each species for each sampling time with all streams included. Note the change in the scale of the y axis for each graph, with the abundance of hydropsychids increasing over time. The abundance of *C. pettiti* decreased from the first to the second sampling time and then rapidly increased in the third sampling time as the number of immature larvae at outlets greatly decreased. At the fourth sampling time there was a very high abundance of *C. pettiti* and an increase in the number of immature larvae downstream. Ontogeny differences would cause individual species to exhibit different patterns through time if early instars could be identified to species

Abundances of larvae between individual streams varied greatly (Table 4.3) with total abundances in Barking Kettle being more than seven times greater than total abundances in Split Rock. Great variation was seen in occurrences and abundances of individual species amongst the streams (Table 4.3) with occurrences of *H. alternans, A. ladogensis* and *D. modesta* restricted to a few of the streams sampled, with reduced abundances compared to the other species. Boxplots depicting the variation in abundances of individual species are shown later in the results. The total number of larvae collected was high providing a substantial data set for testing the derived model.

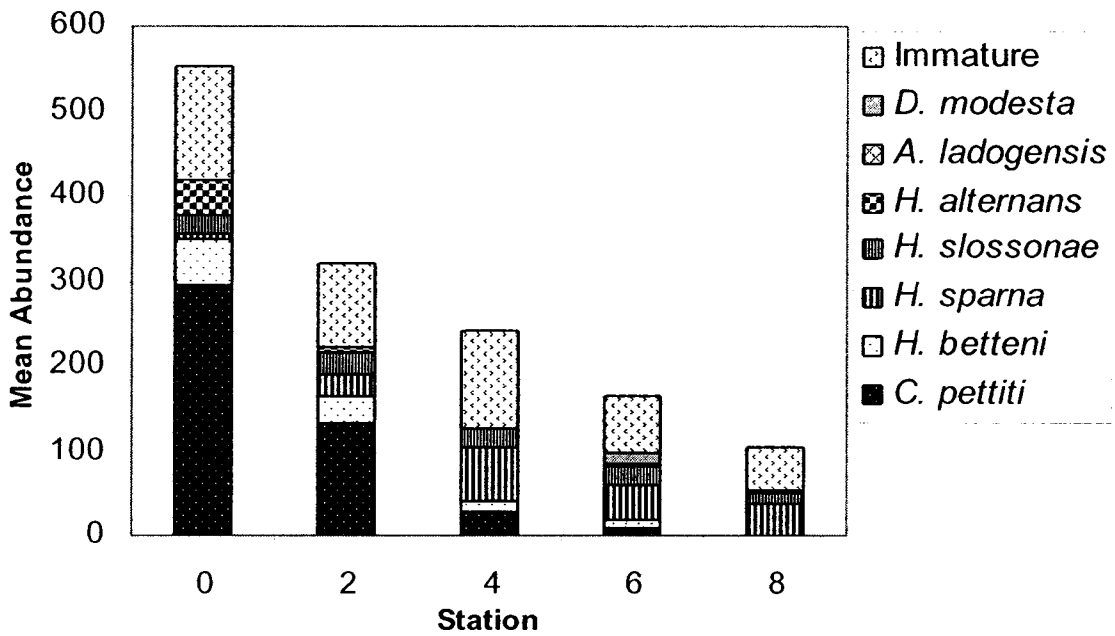

Figure 4.2 Mean abundance of hydropsychids by species from outlets to downstream for all eight streams and all four sampling times.

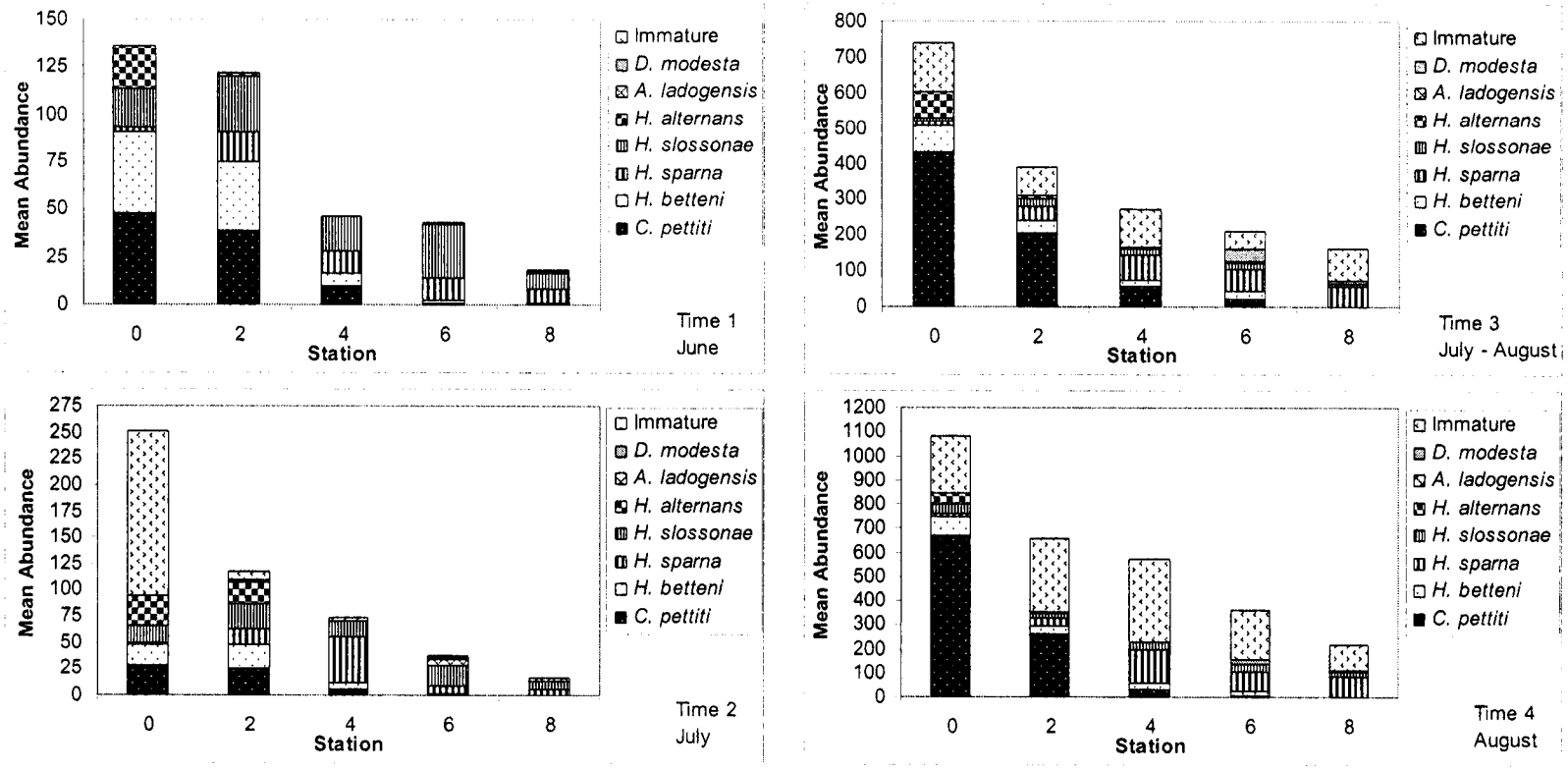

Figure 4.3 Mean abundance of hydropsychid species from outlets to downstream using data collected from the eight streams combined, for each of the four sampling times, with the month(s) of sampling given in the bottom right corner.

Table 4.3 Sum of the number of larvae of each species collected by stream, including the total number of larvae collected by species and by stream, with the four sampling times combined.

	Stream								
Hydrosychidae	Barking Kettle	Great Pond	Broad Cove	Beaver Pond	Split Rock	Above Hatchet	Watern	Portugal Cove	Total
C. pettiti	10162	6250	14696	1683	1	3145	7508	1122	44568
H. betteni	4744	1454	2465	127	1785	0	11	0	10587
H. sparna	428	2518	1492	6392	1650	1638	1000	1754	16872
H. slossonae	2	1659	508	58	0	1365	5403	531	9525
H. alternans	0	0	0	4923	0	0	0	138	5062
A. ladogensis	0	0	0	95	0	0	18	332	445
D. modesta	1504	61	26	0	0	0	0	0	1591
Immature	16330	3183	3340	8745	688	3074	5675	2781	43816
Total	36698	16733	24924	24364	4562	10202	21700	7367	146549

4.3.1.1 Outlet species

Outlet species were defined as those hydropsychid species which most frequently occurred at outlets, often in high abundances. Abundances declined with distance from outlets, with the rate of decline being of particular interest here. In this study, three species occurred most frequently in high abundances at outlets (*C. pettiti, H. betteni, H. alternans*) and dominated the outlet community. Abundances of these three species were summed at each station to analyze the outlet community, hereafter referred to as outlet species. The abundance of outlet species declined significantly with increasing distance downstream ($p<0.0001$) (Figure 4.4), both in forested ($p<0.0001$) and barren ($p<0.0001$) landscapes, during all four sampling times ($p<0.0001$), and in all eight streams ($p<0.0001$) (Figure 4.5). Abundances of outlet species showed considerable variation among streams (Figure 4.5), with Split Rock and Portugal Cove having fewer outlet species overall. The derived model predicted that hydropsychid abundance would decline from the lake outlet at a rate of $e^{-0.5878}$ ($\beta = -0.5878$). Parameter estimates for comparisons to the derived model (Table 4.4) showed outlet species' abundances fit the derived model overall ($\beta = -0.6566$), in only barren landscapes ($\beta = -0.5337$), and over three of the four sampling periods. When streams were considered separately, the model only fit two streams, Great Pond and Watern (Table 4.4). There was a significant interaction by landscape ($p=0.0098$) (Table 4.4), with forested landscapes having a higher abundance and steeper slope than barren landscapes (Figure 4.7 and Figure 4.8 respectively). There was no significant interaction with time ($p=0.4418$) (Figure 4.9).

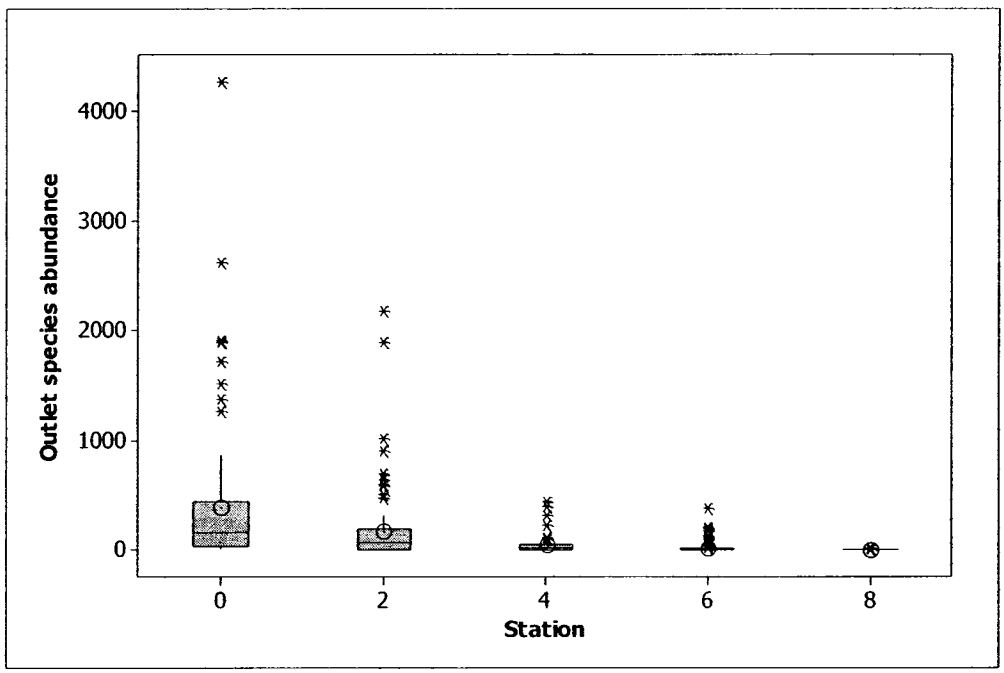

Figure 4.4 Boxplot of outlet species abundance by station. Asterisks indicate outliers, open circles the mean, and boxes are the interquartile range with the line indicating the median.

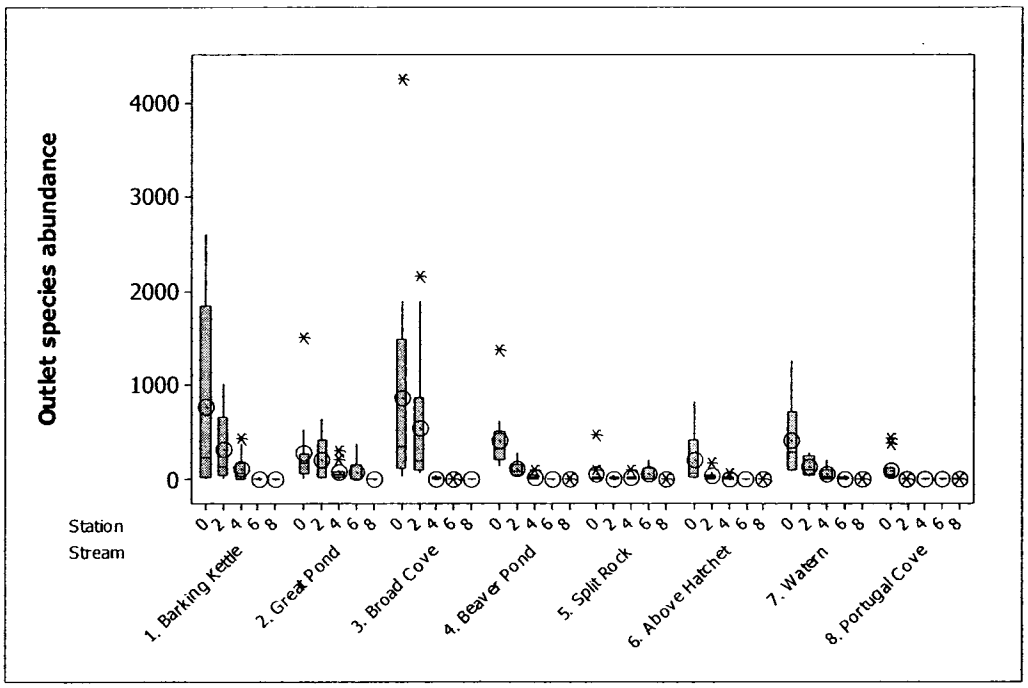

Figure 4.5 Boxplot of outlet species abundance by station for each stream. Asterisks indicate outliers, open circles the mean, and boxes are the interquartile range with the line indicating the median.

Table 4.4 Parameter estimates for outlet species abundance with distance downstream (x); with distance downstream and landscape; with distance downstream and time; and with distance downstream and stream (i=intercept, β=slope).

Variable	n	Parameter	Parameter Estimate	Likelihood Ratio 95% Confidence Limits		Fits model?
x	479	i	6.3331	6.0205	6.6717	yes
		β	-0.6566	-0.7276	-0.5867	
Landscape						
x*forested	239	i	6.8642	6.4829	7.2828	no
		β	-0.7089	-0.7964	-0.6226	
x*barren	240	i	5.3055	4.8503	5.8265	yes
		β	-0.5337	-0.6402	-0.4316	
Sampling time						
x*time1	119	i	5.1628	4.6990	5.6807	yes
		β	-0.6140	-0.7225	-0.5061	
x*time2	120	i	5.1920	4.6875	5.7592	no
		β	-0.7256	-0.8537	-0.6024	
x*time3	120	i	6.7225	6.1348	7.4137	yes
		β	-0.6095	-0.7521	-0.4737	
x*time4	120	i	6.9889	6.3890	7.6932	yes
		β	-0.7079	-0.8501	-0.5698	
Stream						
x*Barking Kettle	60	i	7.1292	6.5511	7.7892	no
		β	-0.7356	-0.8704	-0.6010	
x*Great Pond	60	i	6.0820	5.2286	7.1673	yes
		β	-0.4206	-0.6419	-0.2148	
x*Broad Cove	60	i	8.3337	7.6041	9.1990	no
		β	-1.3937	-1.6346	-1.1782	
x*Beaver Pond	60	i	6.1504	5.6998	6.6539	no
		β	-0.7821	-0.8950	-0.6725	
x*Split Rock	60	i	3.8600	3.1142	4.8249	no
		β	-0.1321	-0.3216	0.0388	
x*Above Hatchet	60	i	5.4400	4.8583	6.1232	no
		β	-0.8326	-0.9970	-0.6821	
x*Watern	60	i	6.3898	5.8475	7.0183	yes
		β	-0.6957	-0.8289	-0.5690	
x*Portugal Cove	60	i	3.8311	2.9484	4.9922	no
		β	-0.9537	-1.3913	-0.6432	

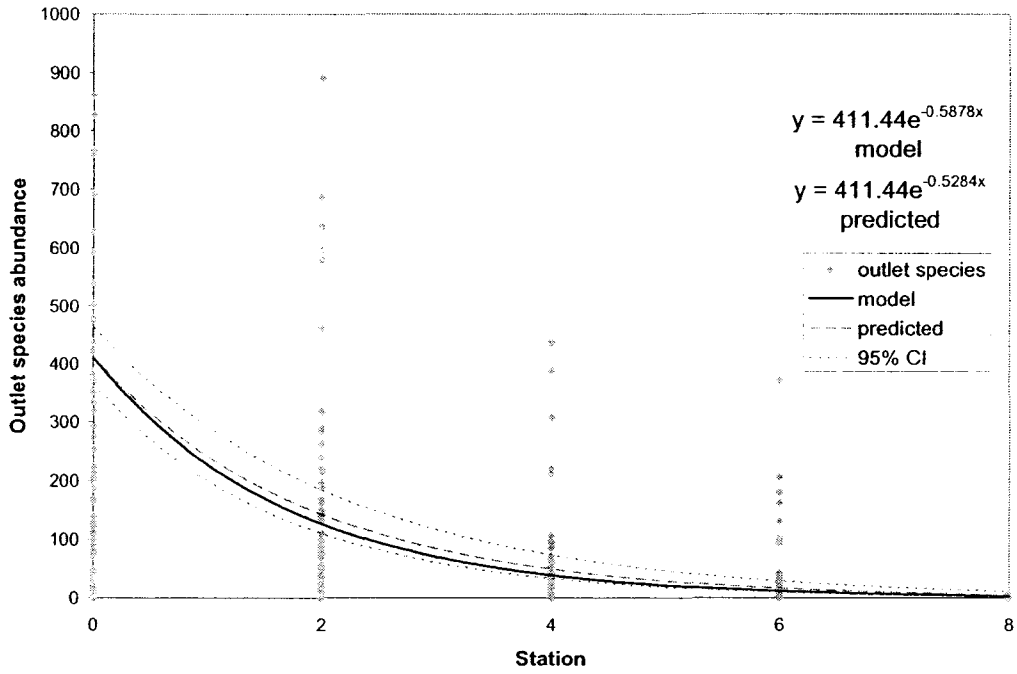

Figure 4.6 Outlet species abundance versus station for all streams and times with the derived model as well as the predicted model with its upper and lower 95% confidence limits.

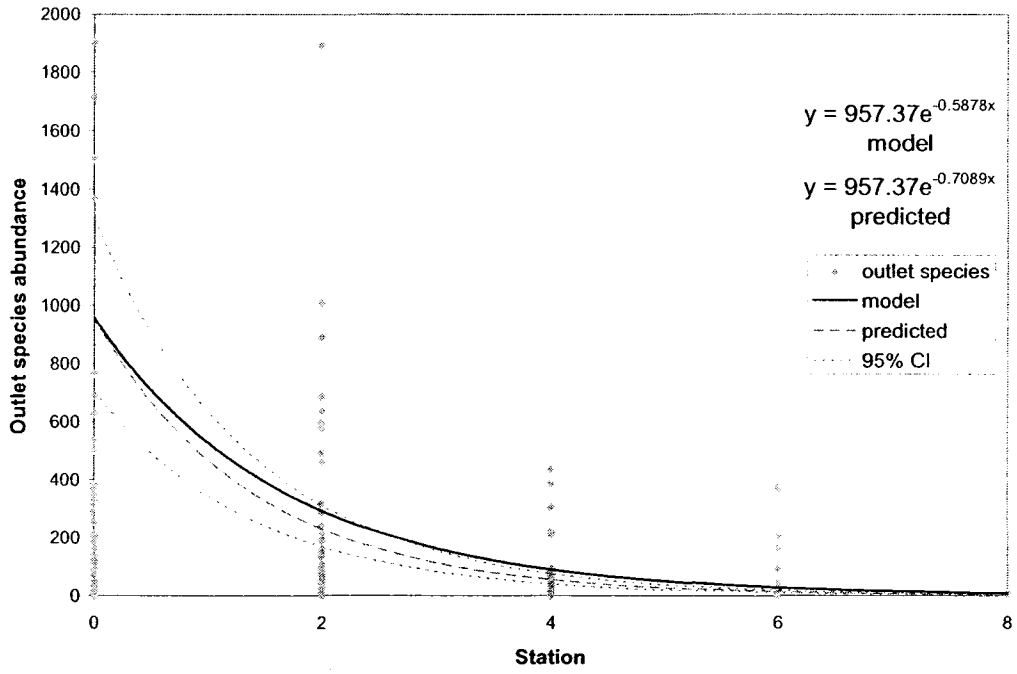

Figure 4.7 Outlet species abundance versus station for forested streams with the derived model as well as the predicted model with its upper and lower 95% confidence limits.

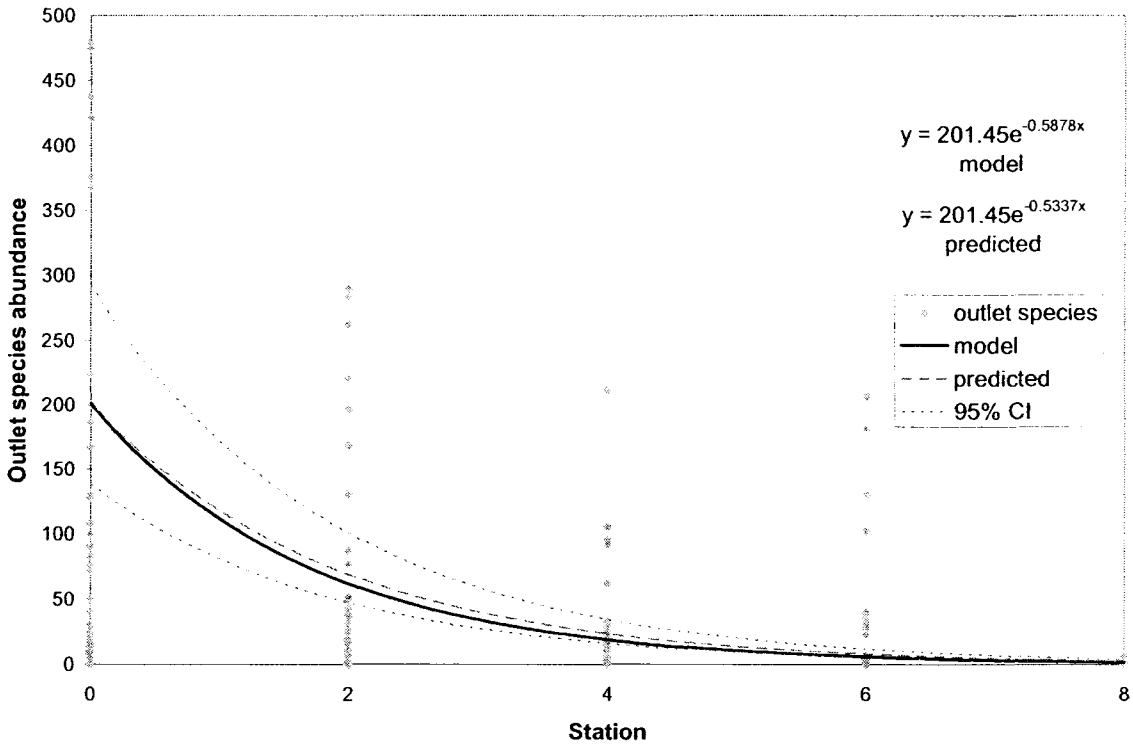

Figure 4.8 Outlet species abundance versus station for barren streams with the derived model as well as the predicted model with its upper and lower 95% confidence limits.

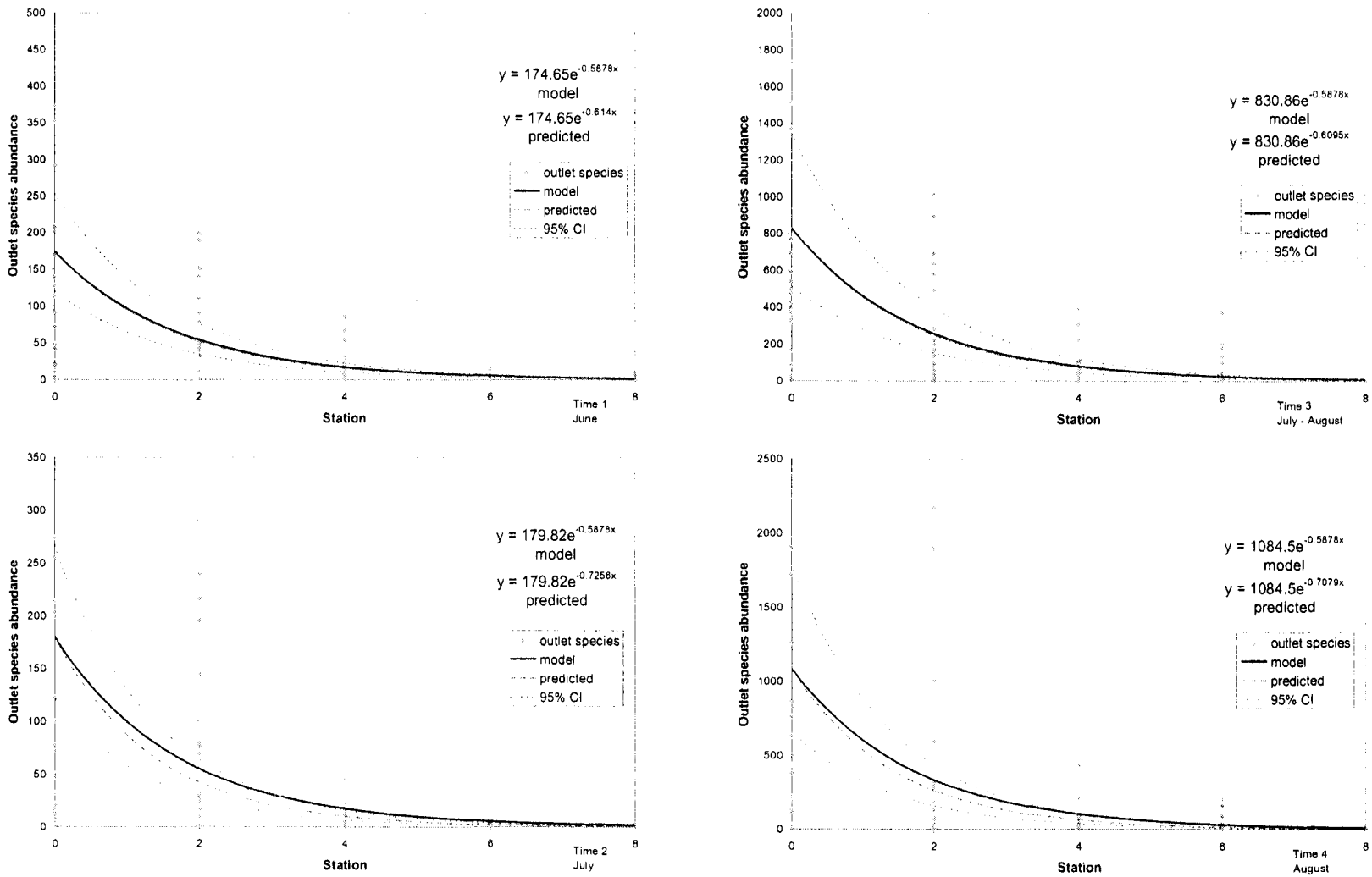

Figure 4.9 Outlet species abundance versus station for each of the sampling times.

4.3.1.2 *Cheumatopsyche pettiti*

Cheumatopsyche pettiti was found in all streams with the exception of Split Rock where it was found during previous sampling (Chapter 2), so Split Rock was excluded for modeling of this species. The abundance of *C. pettiti* showed considerable variation among samples, but a pattern of decline downstream was evident (Figure 4.10); however the nature the profile of the decline among streams was variable with some streams exhibiting a more gradual change in abundance downstream of the outlet than others (Figure 4.11).

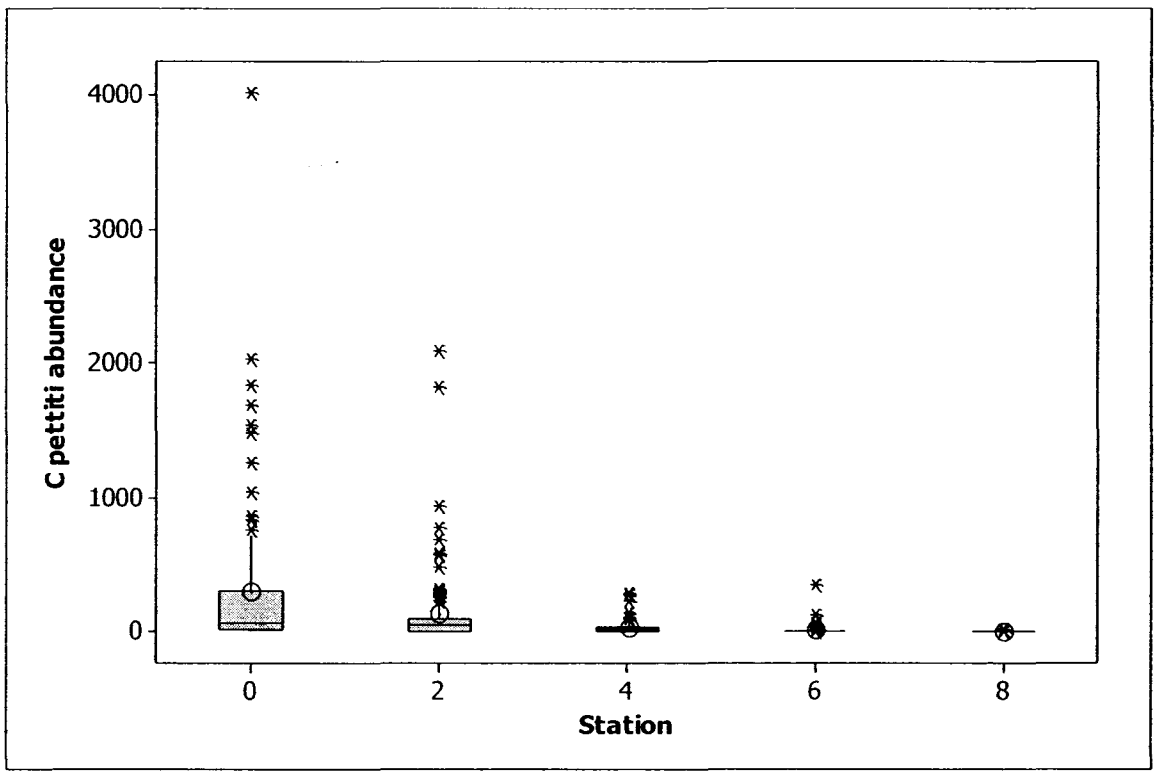

Figure 4.10 Boxplot of *Cheumatopsyche pettiti* abundance from outlets downstream. Asterisks indicate outliers, open circles the mean, and boxes are the interquartile range with the line indicating the median.

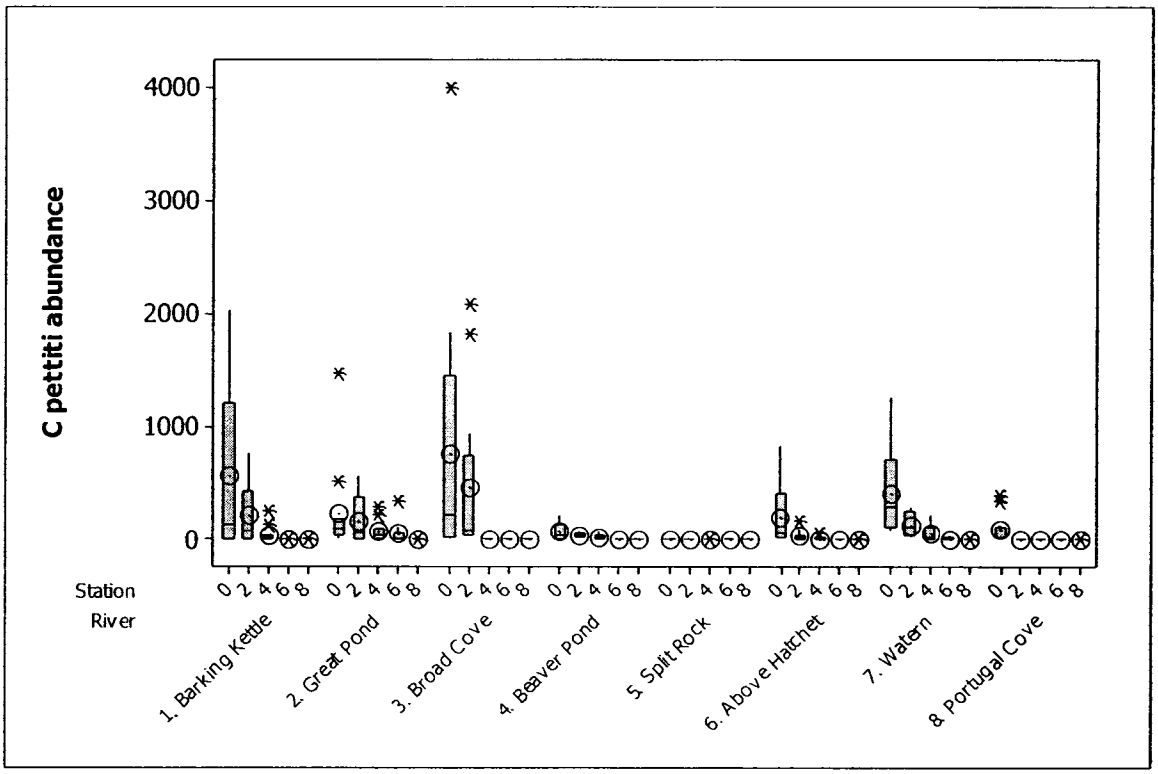

Figure 4.11 Boxplot of *Cheumatopsyche pettiti* abundance by station for each stream. Asterisks indicate outliers, open circles the mean, and boxes are the interquartile range with the line indicating the median.

Regressing the abundance of *C. pettiti* against the distance downstream (x) gave the parameter estimates in Table 4.5. The slope estimate (β= -0.7691) for seven rivers (Split Rock was excluded as only one larva was recovered in this stream (Table 4.3)) over all times was steeper than that of the derived model (β= -0.5878) which did not fit within the predicted 95% confidence limits. The intercept (i) was also estimated, used to determine larval abundance at outlets for comparisons amongst streams and species. Graphing the derived model against the predicted estimates (Figure 4.12) showed the deviance of the model from the real data. The derived model did not fit within the 95% confidence interval of the predicted decay function using the estimated outlet density (e^i),

however the overall abundance of *C. pettiti* from outlets to downstream sites did follow an exponential decay function.

Further exploration of the data showed no significant interaction between the change in abundance with distance downstream and landscape ($p=0.5432$), meaning that the slope estimates did not differ significantly between forested and barren landscapes. Thus, although the abundance of *C. pettiti* in barren landscapes was less, the relative change in abundance with distance downstream followed a similar pattern to that in forested landscapes. There was not a significant interaction between the change in abundance with distance downstream and time ($p=0.6970$), meaning that estimates of the parameters did not differ significantly between sampling times. Although the derived model only fit during the third sampling time, limits of the second sampling time nearly contained the derived slope.

Although the derived model slope was not equivalent to the real data overall, it did hold true for one of the sampling times and for half of the streams as shown by the confidence intervals for β in Table 4.5. The derived model does fit within the confidence limits for Watern (Figure 4.13) and Great Pond (Figure 4.14) over all sampling times.

Table 4.5 Parameter estimates for the abundance of *Cheumatopsyche pettiti* with distance downstream (x); with distance downstream and landscape; with distance downstream and time; and with distance downstream and stream using a log link and a negative binomial distribution (i=intercept, β=slope).

Variable	n	Parameter	Parameter Estimate	Likelihood Ratio 95% Confidence		Fits model?
x	419	i	6.3543	5.9810	6.7635	no
		β	-0.7691	-0.8561	-0.6838	
Landscape						
x*forested	239	i	6.6849	6.1900	7.2443	no
		β	-0.7856	-0.9309	-0.6707	
x*barren	180	i	5.6867	5.1753	6.2722	no
		β	-0.7334	-0.8556	-0.6150	
Sampling time						
x*time1	104	i	4.9215	4.4159	5.5057	no
		β	-0.7489	-0.8918	-0.6194	
x*time2	105	i	4.4249	3.8561	5.0885	no
		β	-0.7379	-0.9019	-0.5902	
x*time3	105	i	6.7741	6.0470	7.6553	yes
		β	-0.7053	-0.8882	-0.5329	
x*time4	105	i	7.0459	6.3806	7.8344	no
		β	-0.8272	-0.9848	-0.6718	
Stream						
x*Barking Kettle	60	i	7.0528	6.2485	8.0112	no
		β	-0.9549	-1.1637	-0.7541	
x*Great Pond	60	i	6.0852	5.1222	7.3501	yes
		β	-0.4712	-0.7285	-0.2369	
x*Broad Cove	60	i	8.9064	7.7478	10.3776	no
		β	-1.8422	-2.3412	-1.4296	
x*Beaver Pond	60	i	5.1426	4.4797	5.9517	yes
		β	-0.7151	-0.9043	-0.5498	
x*Split Rock	60	i	na			
		β				
x*Above Hatchet	60	i	5.4400	4.8583	6.1232	no
		β	-0.8326	-0.9970	-0.6821	
x*Watern	60	i	6.4949	5.9455	7.1317	yes
		β	-0.6974	-0.8318	-0.5692	
x*Portugal Cove	60	i	3.6801	2.6947	5.0787	yes
		β	-0.8829	-1.2637	-0.5775	

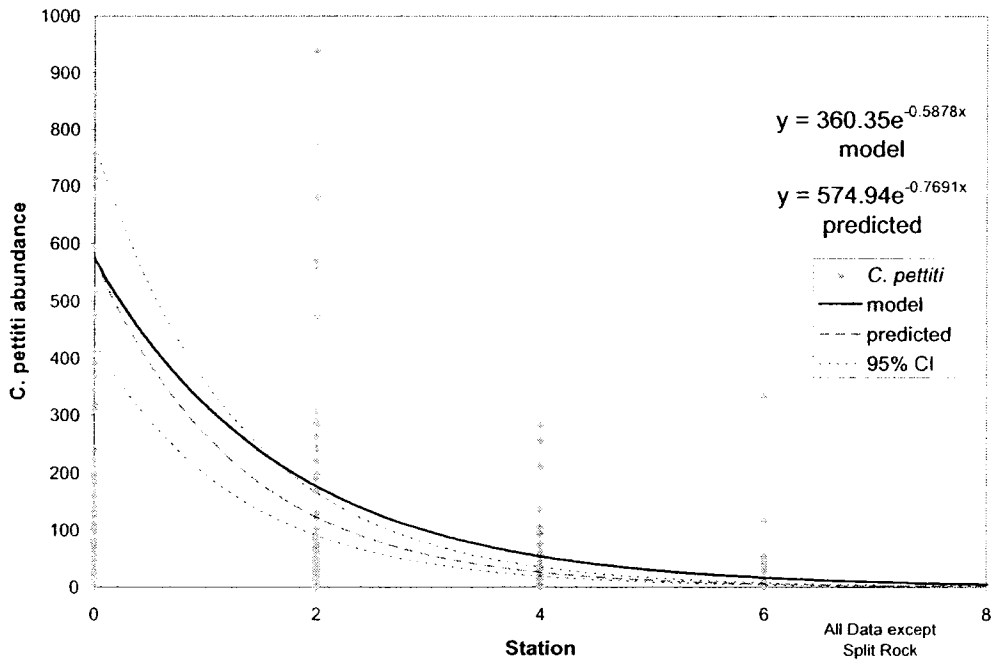

Figure 4.12 *Cheumatopsyche pettiti* abundance versus station for seven streams over all times with the derived model as well as the predicted model with its upper and lower 95% confidence limits.

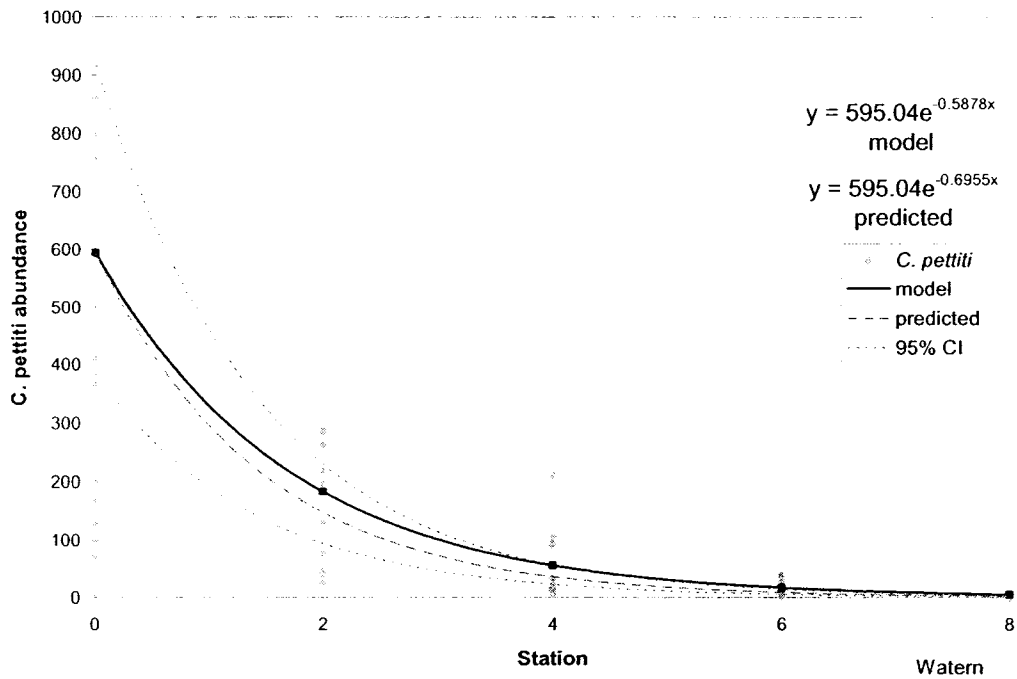

Figure 4.13 *Cheumatopsyche pettiti* abundance versus station for Watern over four sampling times with the derived model and the predicted model with its 95% confidence limits.

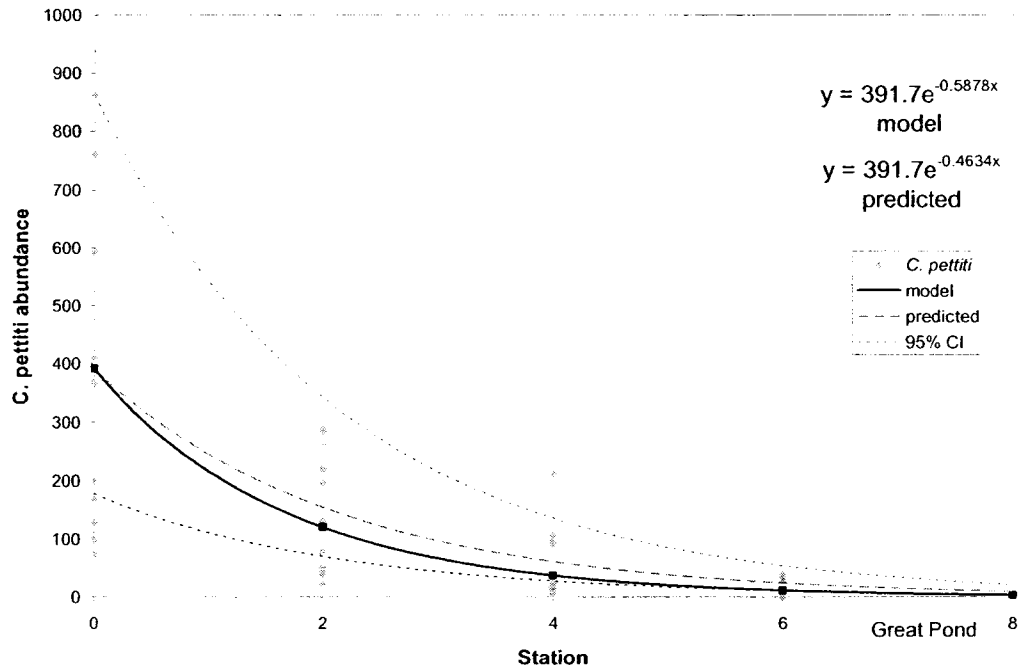

Figure 4.14 *Cheumatopsyche pettiti* abundance versus station for Great Pond over all sampling times with the derived model and the predicted model with its 95% confidence limits.

4.3.1.3 *Hydropsyche betteni*

The mean abundance of *H. betteni* generally followed a decay function from the outlet to downstream in the five streams where it was recovered in sufficient abundances for modeling. Above Hatchet, Watern and Portugal Cove yielded too few individuals (Figure 4.16; Table 4.3) to be included in the analysis. Its outlet abundance was lower, but it was found in higher numbers further downstream than *C. pettiti*; however, there were many outliers (Figure 4.15). It was mainly collected in the four forested streams (Barking Kettle, Broad Cove, Beaver Pond and Great Pond) where it showed a general decline in abundance from the outlet. In Split Rock, a stream in a barren landscape, its distribution was quite variable throughout (Figure 4.16).

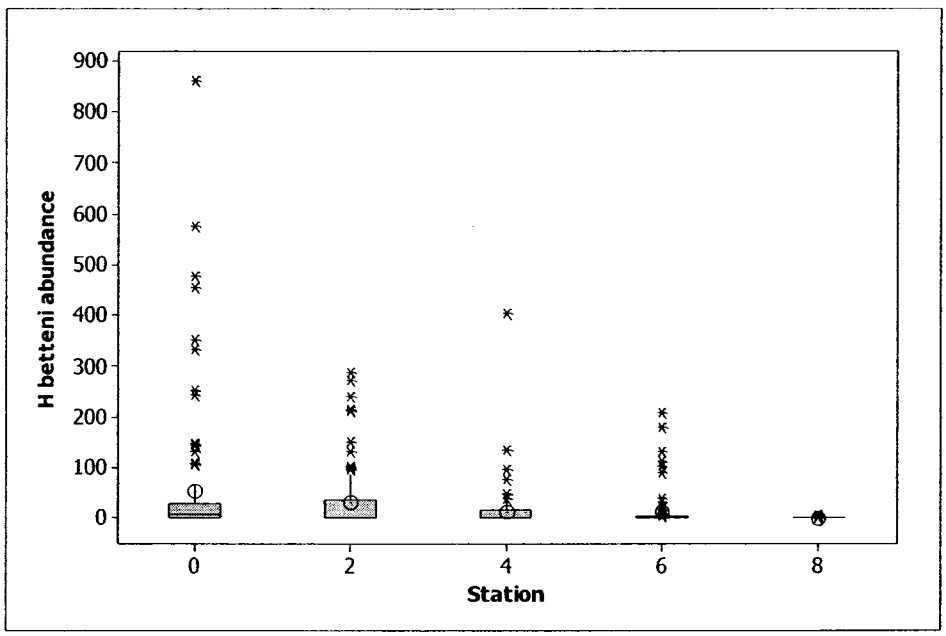

Figure 4.15 Boxplot of *Hydropsyche betteni* abundance from outlets downstream for all streams and sampling times. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

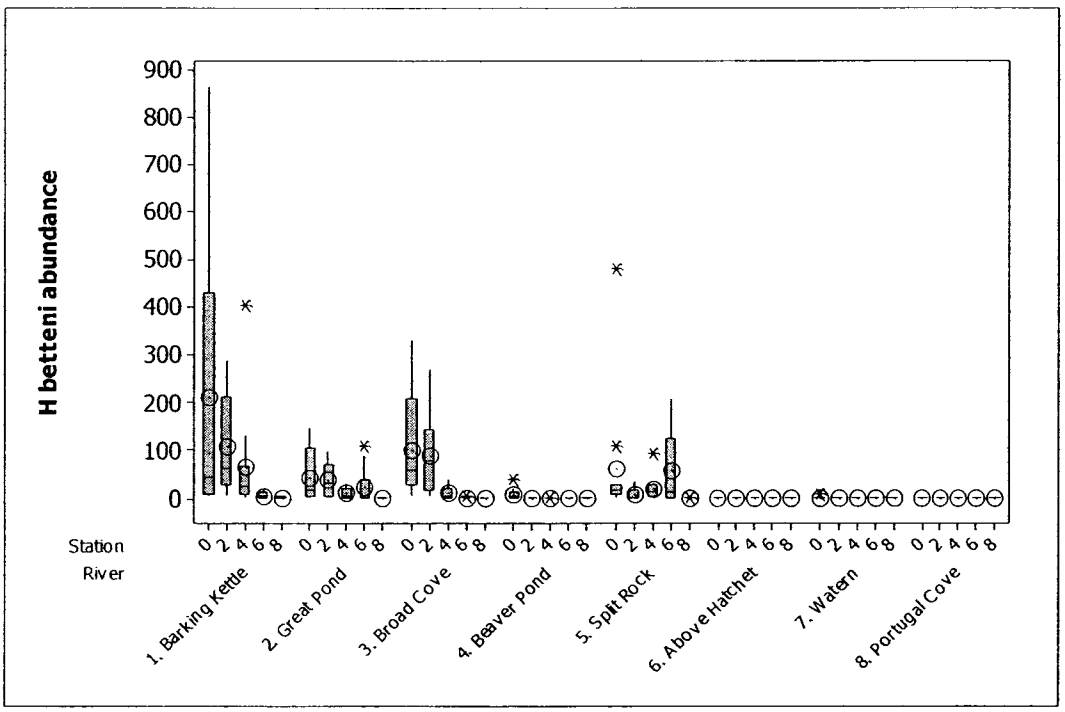

Figure 4.16 Boxplot of *Hydropsyche betteni* abundance by station for each stream over all times. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

Regressing the abundance of *H. betteni* against the distance downstream (x) gave the parameter estimates in Table 4.6. The estimate of the slope using five streams over all times (β=-0.4242) was more gradual than the derived model (β=-0.5878) which did not fall within the predicted 95% confidence limits for this parameter. Using the predicted intercept and graphing the slope of the derived model against that of the predicted model (Figure 4.17) showed the deviance of the model from the data. Thus the model slope did not fit the overall decay pattern of *H. betteni* from an outlet downstream.

There was a significant interaction between the change in abundance with distance downstream and landscape (p=0.0008), meaning that the estimates of the parameters differed significantly between forested and barren landscapes. In forested landscapes the derived slope fell within the 95% confidence limits of the predicted slope (Figure 4.18, Table 4.6). In barren landscapes the change in abundance with distance downstream was less, resulting in a gentler slope that was significantly different from that of the derived model. However, this species could only be modeled in one barren stream (Table 4.6). This means that the abundance of *H. betteni* may differ with landscape.

There was not a significant interaction between the change in abundance with distance downstream and time (p=0.1836), meaning that the estimates of the parameters did not differ significantly between sampling times. Even with the lower abundance at outlets during the second sampling regime, the slopes of the change in abundance with distance over time were not significantly different; however time 3 and 4 had a gentler slope as shown by the parameter estimates (Table 4.6). The 95% confidence limits for the

slope parameters for all but time 3 did fit the derived model, and were close for time 1 and time 2 which had steeper rates of decline.

There was a significant interaction ($p<0.0001$) between the change in slope and stream. There was a highly significant change ($\alpha<0.001$) in the abundance of *H. betteni* with distance downstream for all forested streams. However, for Split Rock this change was not significant ($p=0.1354$). The model slope only fell within the 95% confidence limits in Barking Kettle, where slopes were quite similar (Figure 4.19).

Table 4.6 Parameter estimates for the abundance of *Hydropsyche betteni* with distance downstream (x); with distance downstream and landscape; with distance downstream and time; and with distance downstream and stream using a log link and a negative binomial distribution (i=intercept, β=slope).

Variable	n	Parameter	Parameter Estimate	Likelihood Ratio 95% Confidence		Fits model?
x	299	i	4.7532	4.3343	5.2217	no
		β	-0.4242	-0.5218	-0.3293	
Landscape						
x*forested	239	i	5.0176	4.5552	5.5343	yes
		β	-0.5458	-0.6538	-0.4396	
x*barren	60	i	3.8594	3.1036	4.8412	no
		β	-0.1321	-0.3247	0.0413	
Sampling time						
x*time1	74	i	4.7236	4.0918	5.4597	yes
		β	-0.5748	-0.7261	-0.4246	
x*time2	75	i	4.2690	3.6227	5.0201	yes
		β	-0.5691	-0.7316	-0.4124	
x*time3	75	i	4.9096	4.1427	5.8764	no
		β	-0.3555	-0.5488	-0.1758	
x*time4	75	i	5.0238	4.1001	6.2426	yes
		β	-0.3931	-0.6368	-0.1710	
Stream						
x*Barking Kettle	60	i	5.8225	5.2407	6.4880	yes
		β	-0.5719	-0.7096	-0.4359	
x*Great Pond	60	i	4.2277	3.4261	5.2339	no
		β	-0.2996	-0.5009	-0.1100	
x*Broad Cove	60	i	5.8900	5.2017	6.7281	no
		β	-0.9605	-1.1794	-0.7726	
x*Beaver Pond	60	i	2.2042	1.4480	3.1663	no
		β	-1.0767	-1.5310	-0.7203	
x*Split Rock	60	i	3.8594	3.1036	4.8412	no
		β	-0.1321	-0.3247	0.0413	
x*Above Hatchet	60	i	na			
		β				
x*Watern	60	i	na			
		β				
x*Portugal Cove	60	i	na			
		β				

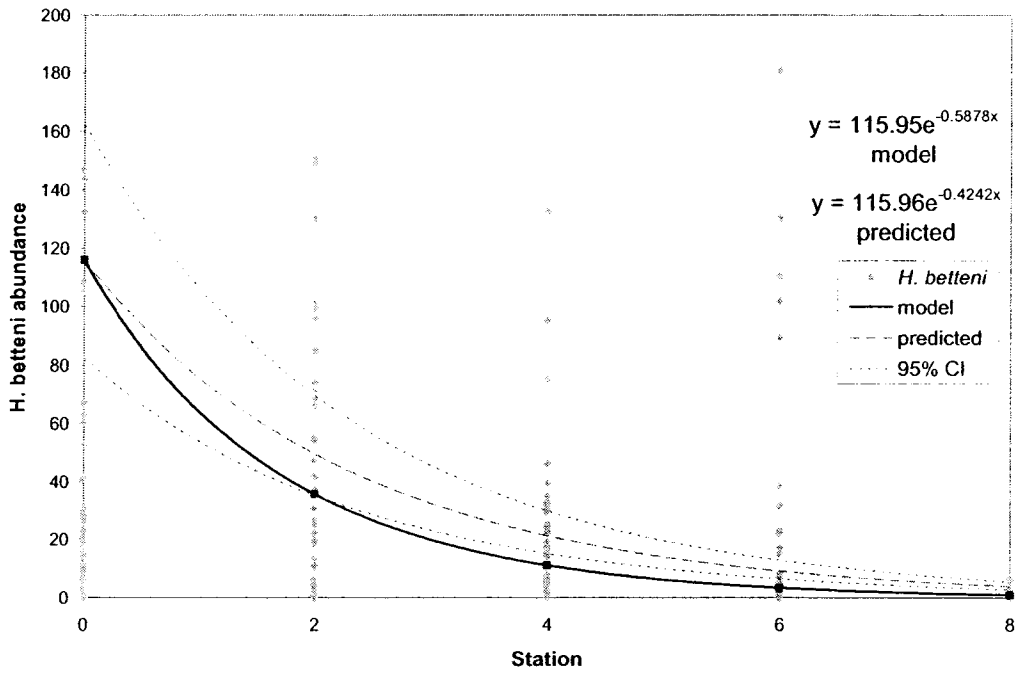

Figure 4.17 *Hydropsyche betteni* abundance versus station for five streams over all times with the derived model as well as the predicted model with its upper and lower 95% confidence limits.

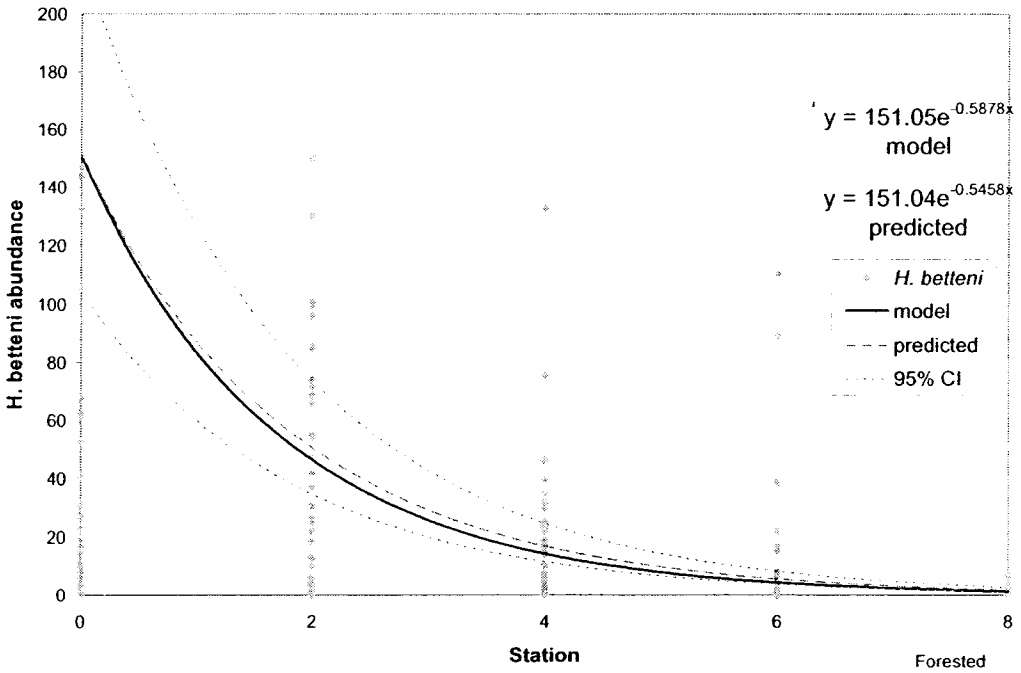

Figure 4.18 *Hydropsyche betteni* abundance versus station for forested streams over all times with the derived model as well as the predicted model with its upper and lower 95% confidence limits.

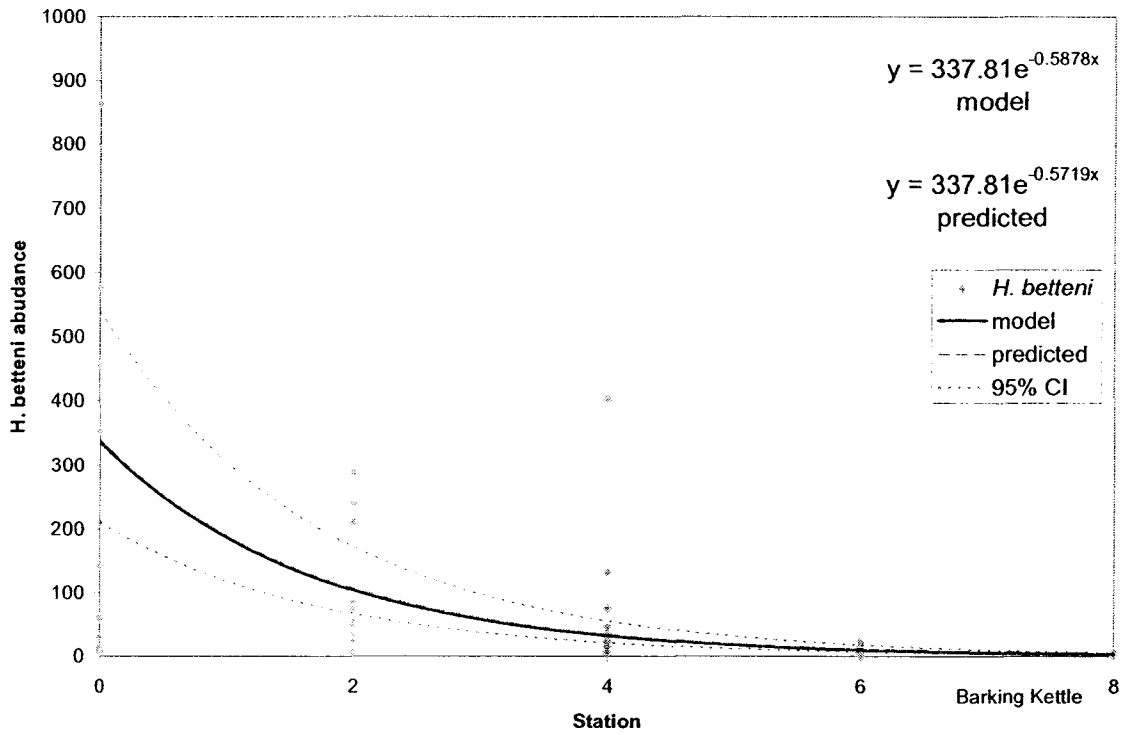

Figure 4.19 *H. betteni* abundance versus station for Barking Kettle over all times, with the derived model and the predicted model with its 95% confidence limits.

4.3.1.4 *Hydropsyche alternans*

Hydropsyche alternans occurred in only two of the eight streams, near outlets (Figure 4.20). Abundances in Portugal Cove were too low to test against the derived model, but in Beaver Pond this species generally followed a decay pattern. After removal of the outlier of ~1200, the slope estimate of *H. alternans* abundance in Beaver Pond (β= -0.8251 [-1.0180, -0.6609]) was found to significantly differ from the slope of the derived model based on the estimated intercept (i=5.6013 [5.3780, 5.8098]) where the 95% confidence limits are in square brackets.

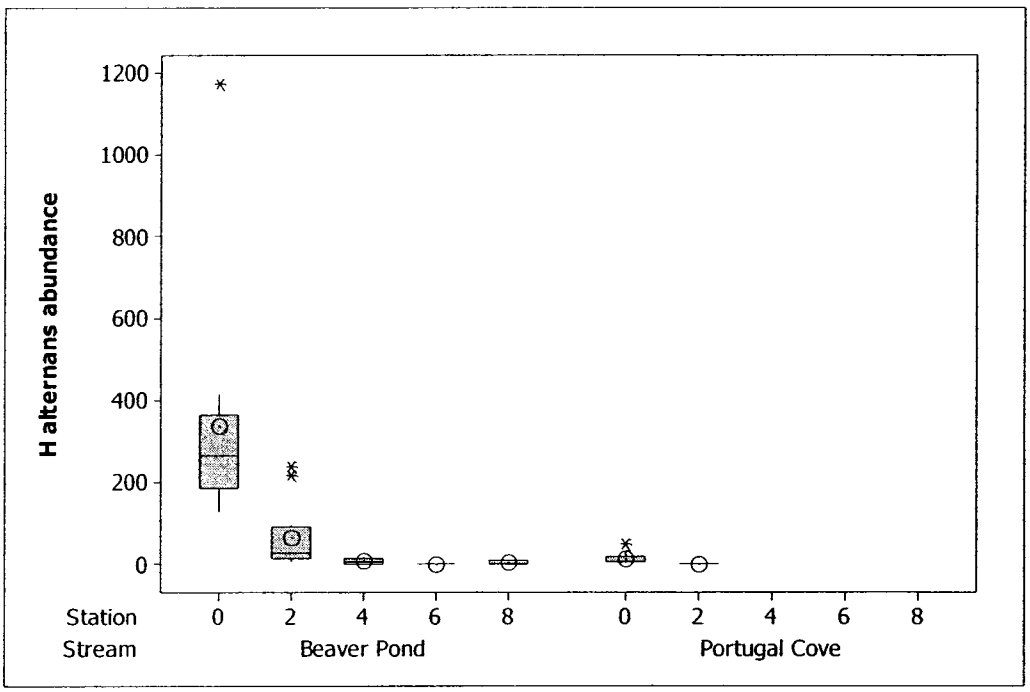

Figure 4.20 Boxplot of *Hydropsyche alternans* abundance by station in the two streams where it occurred over all sampling times. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

4.3.1.5 Downstream species

Downstream communities generally consisted of four species (*H. slossonae, H. sparna, A. ladogensis, D. modesta*) which were considered as a whole when examining patterns of abundance in the downstream community. The sum of abundances of these four species is hereafter referred to as downstream species abundance. Abundances generally increased from outlets to station six and declined slightly by station eight (Figure 4.21). However, Watern and Portugal Cove generally declined and Beaver Pond had a pronounced peak at station four (Figure 4.22). Using a GLzM with an identity link and a negative binomial distribution it was found that their abundance did not significantly change from outlets to downstream over all eight rivers and four sampling

times ($p=0.7487$, Table 4.7). There was not a significant interaction with their downstream abundance and landscape ($p=0.5026$, Table 4.7). Abundances in barren ($p=0.8638$, Table 4.7) and forested ($p=0.5300$, Table 4.7) streams remained relatively constant with station, with forested streams having higher abundances. There was not a significant interaction with time ($p=0.2141$), although abundances generally increased over time with sampling times 3 and 4 having a positive slope indicating greater abundances further downstream (Table 4.7). There were also significant differences in abundance with station amongst the streams sampled, with Barking Kettle, Broad Cove and Above Hatchet having significant (all $p<0.0001$) increases downstream, whereas abundances declined downstream in Portugal Cove ($p=0.0181$) and Watern ($p=0.0093$) (Table 4.7).

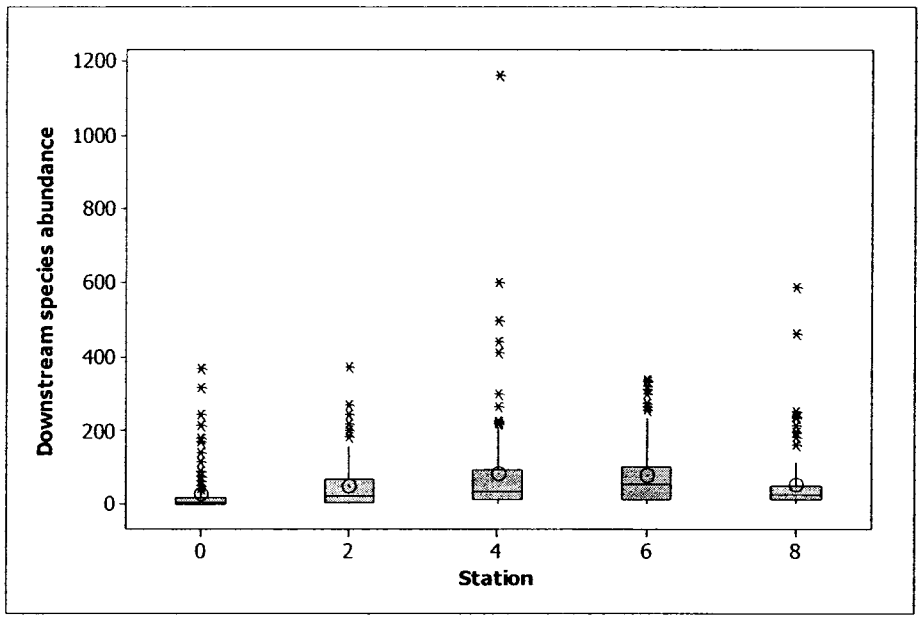

Figure 4.21 Boxplot of downstream species abundance by station including all streams and sampling times. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

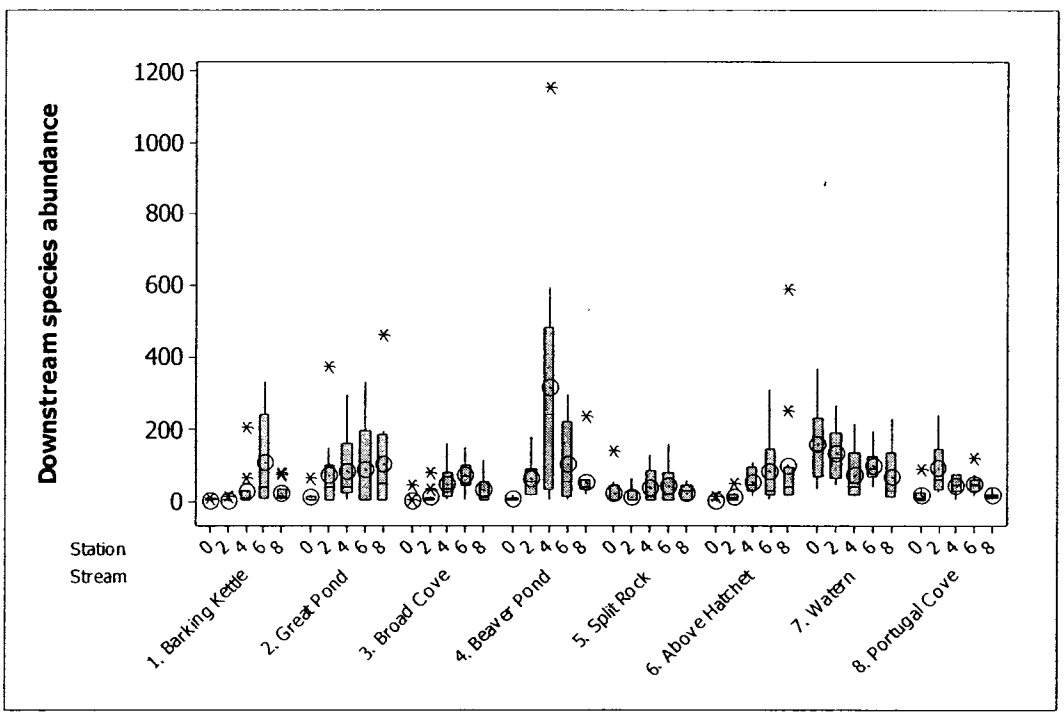

Figure 4.22 Boxplot of downstream species abundance by station for each stream over all sampling times. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

Table 4.7 Parameter estimates for the downstream species abundance of hydropsychids with distance downstream (x); with distance downstream and landscape; with distance downstream and time; and with distance downstream and stream using an identity link and a negative binomial distribution (i=intercept, β=slope).

Variable	n	Parameter	Parameter Estimate	Likelihood Ratio 95% Confidence	
x	479	i	28.0049	47.0710	71.2600
		β	0.0432	-0.1927	0.3775
Landscape					
x*forested	239	i	56.0028	27.4782	81.2959
		β	0.1884	-0.2899	1.9464
x*barren	240	i	27.7722	45.8827	73.2633
		β	-0.0231	-0.2566	0.2928
Sampling time					
x*time1	119	i	36.4087	25.8097	52.6208
		β	-0.1724	-0.3470	0.0483
x*time2	120	i	40.1883	27.0706	61.9150
		β	-0.2166	-0.4377	0.0515
x*time3	120	i	58.7091	38.5481	84.0921
		β	0.4391	-0.1389	1.5463
x*time4	120	i	90.2331	56.8316	137.7020
		β	0.3729	-0.4519	2.0951
Stream					
x*Barking Kettle	60	i	0.7075	0.2147	2.9419
		β	1.6255	0.9249	3.1669
x*Great Pond	59	i	54.4543	27.3306	90.8119
		β	0.5505	-0.1483	1.4690
x*Broad Cove	60	i	5.3614	2.1032	20.5046
		β	1.8459	0.5268	3.3884
x*Beaver Pond	60	i	130.4626	83.3474	210.5966
		β	-0.6478	-1.4779	0.6134
x*Split Rock	60	i	26.7572	15.3343	41.5262
		β	0.0235	-0.214	0.3440
x*Above Hatchet	60	i	2.8342	1.3687	6.6153
		β	2.5121	1.6867	3.7504
x*Watern	60	i	122.609	96.5815	157.9490
		β	-0.5005	-0.9016	-0.0437
x*Portugal Cove	60	i	55.138	42.7174	72.4272
		β	-0.3586	-0.5304	-0.1969

4.3.1.6 *Hydropsyche sparna*

Hydropsyche sparna occurred in all streams and had low abundances at outlets, reached its highest abundance in the mid reaches and declined slightly further downstream (Figure 4.23), with considerable variation amongst streams (Figure 4.24). Longitudinal changes in abundances of *H. sparna* clearly did not follow a negative power function and thus this species could not be compared to the derived model. Instead a GLzM model with an identity link and a negative binomial distribution was an adequate model when the outlier of ~1200 was removed. There was no significant change in *H. sparna* abundance with increasing distance downstream ($p=0.0795$), nor was there an interaction with landscape ($p=0.9503$, Table 4.8) meaning that changes in abundances with station were similar in both forested and barren landscapes. Abundances remained relatively constant with downstream distance in both forested ($p=0.4503$) and barren ($p=0.0541$) landscapes, with slightly higher abundances in forested landscapes (Table 4.8). There was a significant interaction with time ($p=0.0341$), where time one and time two had decreased abundances downstream and time three and four increased (Table 4.8), but this change was only significant for time four ($p=0.0371$). There was also a significant interaction amongst streams ($p<0.0001$), with all streams except Split Rock, Watern and Portugal Cove having a significant increase downstream ($p<0.0206$). Portugal Cove was the only stream showing a significant decrease downstream ($p<0.0001$) (Table 4.8).

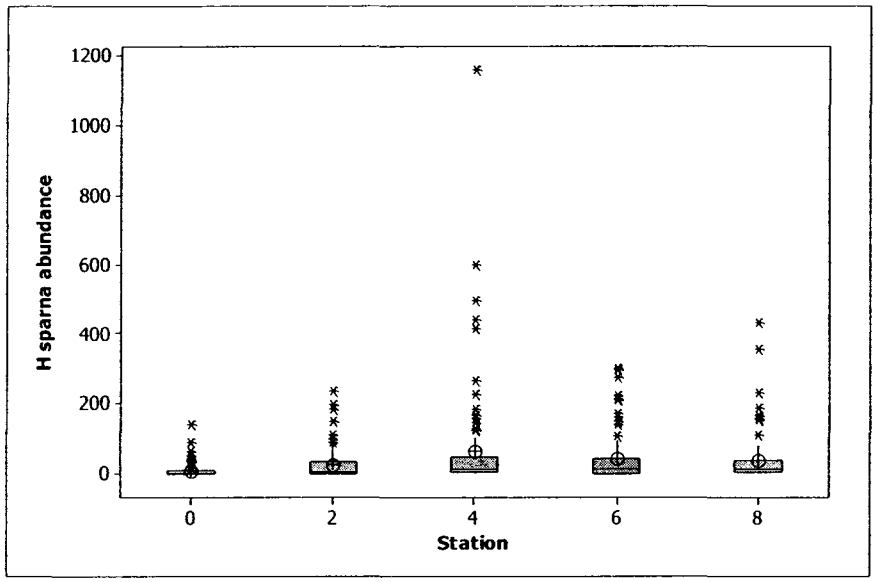

Figure 4.23 Boxplot of *Hydropsyche sparna* abundance from outlets downstream. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

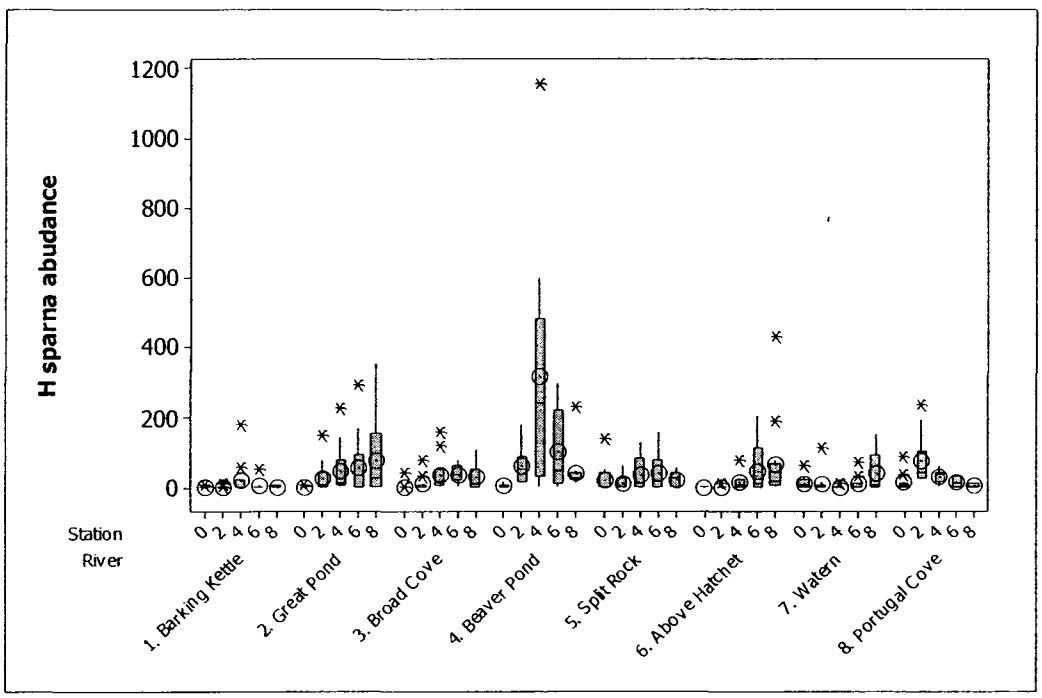

Figure 4.24 Boxplot of *Hydropsyche sparna* abundance by station for each stream. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

Table 4.8 Parameter estimates for *Hydropsyche sparna* abundance with distance downstream (x); with distance downstream and landscape; with distance downstream and time; and with distance downstream and stream using an identity link and a negative binomial distribution (i=intercept, β=slope).

Variable	n	Parameter	Parameter Estimate	Likelihood Ratio 95% Confidence	
x	478	i	27.6097	20.6323	36.1126
		β	0.1747	-0.0170	0.493
Landscape					
x*forested	238	i	36.1010	19.0610	54.0345
		β	0.1501	-0.1791	1.1974
x*barren	240	i	20.0496	14.3885	28.0273
		β	0.1656	-0.0025	0.4360
Sampling time	478				
x*time1	119	i	10.2797	6.9140	15.5299
		β	-0.0175	-0.0831	0.0904
x*time2	120	i	18.0802	10.5002	34.0167
		β	-0.1107	-0.2625	0.0652
x*time3	120	i	39.3528	24.8097	59.2957
		β	0.2761	-0.1227	1.0994
x*time4	119	i	37.4636	17.1183	64.9134
		β	0.8183	0.0341	2.7581
Stream					
x*Barking Kettle	60	i	8.5343	3.2985	21.1042
		β	-0.0426	-0.1636	0.0708
x*Great Pond	59	i	21.9723	5.8802	45.6886
		β	0.6563	0.1168	1.3697
x*Broad Cove	60	i	9.0374	2.3374	30.5440
		β	0.8191	-0.0426	2.3799
x*Beaver Pond	59	i	106.2985	66.8320	173.0012
		β	-0.5212	-1.2167	0.5957
x*Split Rock	60	i	26.7572	15.3343	41.5262
		β	0.0235	-0.2140	0.3440
x*Above Hatchet	60	i	0.8934	0.3530	2.4073
		β	1.0038	0.6460	1.6267
x*Watern	60	i	9.3078	5.1948	17.9647
		β	0.2058	0.0350	0.5822
x*Portugal Cove	60	i	38.1816	27.4876	54.9909
		β	-0.3009	-0.4560	-0.1927

4.3.1.7 *Hydropsyche slossonae*

Abundances of *H. slossonae* changed very little with distance from outlets (Figure 4.25). No specimens were found in Split Rock and very few in Barking Kettle and Beaver Pond (Figure 4.26) and so these streams could not be included in the modeling. Abundances amongst streams varied greatly (Figure 4.26). Thus changes in *H. slossonae* abundances below outlets did not conform to a negative power function model. Instead, regression was carried out using a GLzM with an identity link and negative binomial distribution as this was an appropriate model for these data.

The downstream distribution of *H. slossonae* significantly decreased using all five rivers over all four sampling times ($p=0.0097$, Table 4.9). There was not a significant interaction with landscape ($p=0.2105$) and abundances did not change with downstream distance in forested landscapes ($p=0.3118$) but did in barren landscapes ($p=0.0121$) which also had higher abundances (Table 4.9). There was not a significant interaction with time ($p=0.6584$), with time one ($p=0.0101$) and time two ($p=0.0201$) having significant decreases in abundances downstream. This trend continued over time three and four but was not significant (Table 4.9). There were significant differences amongst the five streams ($p<0.0001$). Abundances in Great Pond, Broad Cove and Portugal Cove did not differ with downstream distance, but abundances decreased in Watern ($p<0.0001$) and increased in Above Hatchet ($p=0.005$) (Table 4.9).

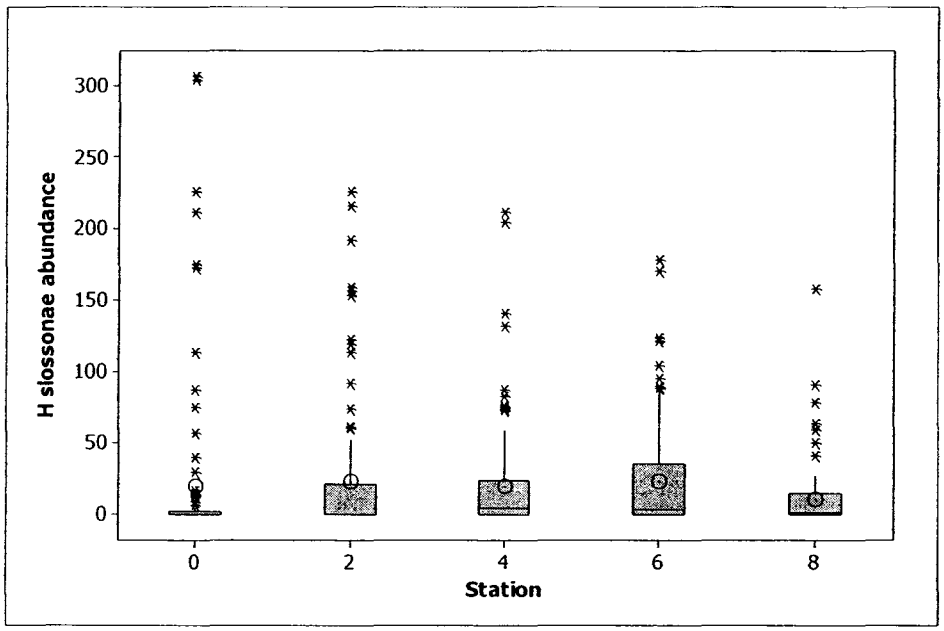

Figure 4.25 Boxplot of *Hydropsyche slossonae* abundance from outlets downstream. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

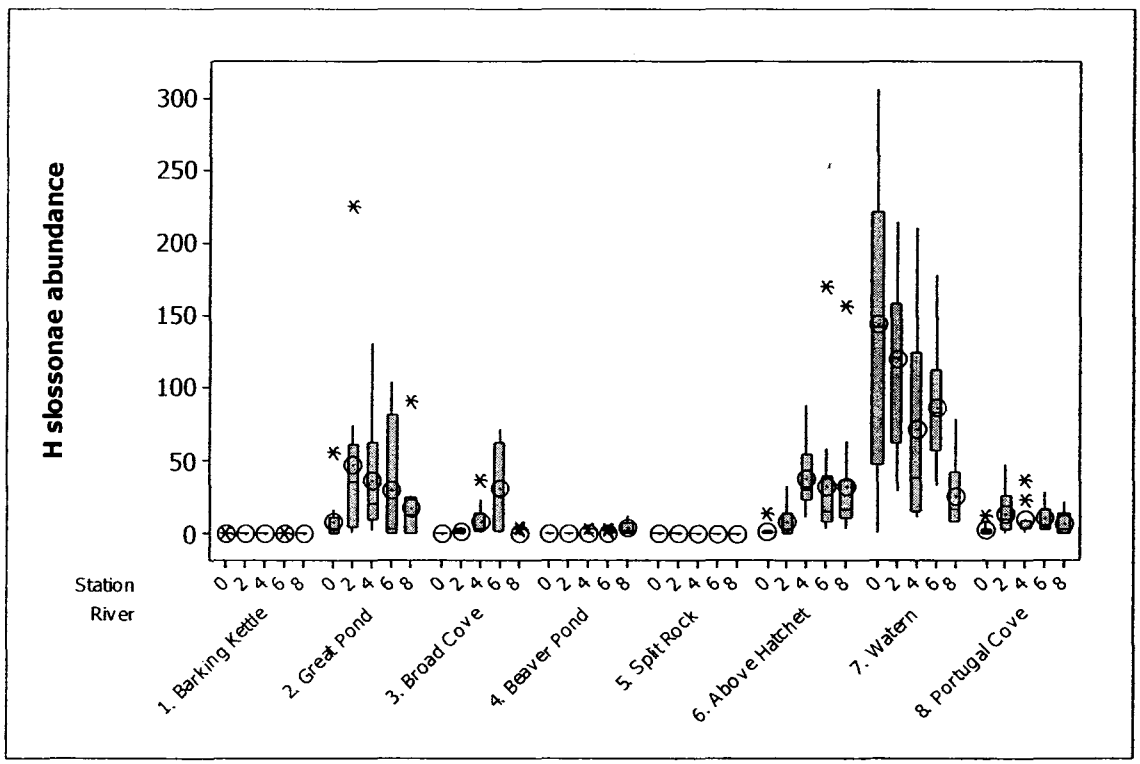

Figure 4.26 Boxplot of *Hydropsyche slossonae* abundance by station for each stream. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

Table 4.9 Parameter estimates for *Hydropsyche slossonae* abundance with distance downstream (x); with distance downstream and landscape; with distance downstream and time; and with distance downstream and stream using an identity link and a negative binomial distribution (i=intercept, β=slope).

Variable	n	Parameter	Parameter Estimate	Likelihood Ratio 95% Confidence	
x	299	i	37.5209	29.8577	47.6920
		β	-0.1821	-0.2958	-0.0537
Landscape					
x*forested	119	i	21.4679	12.9886	35.5483
		β	-0.0971	-0.2483	0.1808
x*barren	180	i	48.0493	37.5918	62.3272
		β	-0.2365	-0.3923	-0.0638
Sampling time	299				
x*time1	74	i	42.1329	29.8903	61.2832
		β	-0.2744	-0.4619	-0.0919
x*time2	75	i	32.3637	23.2070	43.4673
		β	-0.2007	-0.3321	-0.0368
x*time3	75	i	26.3057	16.8627	42.0987
		β	-0.1077	-0.2796	0.1621
x*time4	75	i	49.2201	30.3574	73.6993
		β	-0.1429	-0.4743	0.2983
Stream					
x*Barking Kettle	60	i	na		
		β			
x*Great Pond	59	i	31.7374	19.7874	47.1238
		β	-0.1148	-0.3181	0.1591
x*Broad Cove	60	i	10.1770	1.4981	18.5324
		β	-0.0543	-0.1625	0.2454
x*Beaver Pond	59	i	na		
		β			
x*Split Rock	60	i	na		
		β			
x*Above Hatchet	60	i	2.1214	0.8455	7.7338
		β	1.4561	0.6970	2.3653
x*Watern	60	i	115.4255	92.1421	146.8307
		β	-0.8163	-1.1184	-0.5573
x*Portugal Cove	60	i	9.5260	6.4642	14.2134
		β	-0.0212	-0.0791	0.0704

4.3.1.8 *Arctopsyche ladogensis* and *Diplectrona modesta*

Arctopsyche ladogensis and *D. modesta* had low abundances (Figure 4.2 & Figure 4.3, Table 4.3) across all streams and stations, with each only occurring in three of the

eight steams. Abundances of *A. ladogensis* were generally low (Table 4.3) as were those of *D. modesta* except for Station 6 in Barking Kettle (Table 4.3) and could not be modeled.

4.3.2 Physiochemistry, plankton and periphyton

The pH, conductivity, temperature, velocity, phytoplankton, periphyton and zooplankton measurements were regressed against the overall abundance of hydropsychids as well as against the abundance of each species, with significant p values (α=0.05) given in Table 4.10. However, plotting the abundance of each species against these variables did not reveal clear relationships because the hydropsychid data were highly variable and regression correlations were weak.

Table 4.10 Significance (p values) of linear relationships between species and factors (physical, chemical and nutrients) with the adjusted r^2 value for the multiple linear regressions of these dependent variables against each species.

Factor	*C. pettiti*	*H. betteni*	*H. sparna*	*H. slossonae*	*H. alternans*	*A. ladogensis*	*D. modesta*	Immature	Total
pH	ns	<0.0001	ns	ns	<0.0001	ns	<0.0001	ns	ns
Conductivity	<0.0001	0.0050	0.0050	0.0242	<0.0001	ns	ns	0.0383	ns
Temperature	<0.0001	<0.0001	<0.0001	ns	ns	ns	<0.0001	ns	<0.0001
Velocity	ns	0.0001	<0.0001	0.0004	<0.0001	ns	ns	ns	ns
Periphyton	ns	0.0020	<0.0001	ns	<0.0001	<0.0001	ns	ns	ns
Phytoplankton	ns	0.0152	0.0002	ns	ns	ns	ns	0.0001	0.0016
Zooplankton	ns	0.0101	0.0438	ns	<0.0001	ns	<0.0001	0.0006	<0.0001
r^2 adjusted (%)	25.64	9.47	9.53	6.00	9.51	2.36	11.49	5.20	18.68

A principal component analysis (PCA) was conducted using pH, conductivity, temperature, abundances of periphyton, phytoplankton, zooplankton and abundances of each species of hydropsychid. The data were standardized in order to compare values with differing units. The correlation matrix in Table 4.11 showed weak correlations amongst the variables. The factor loadings plot (Figure 4.27) showed *C. pettiti* and *H. betteni* were closely associated with each other and with zooplankton. *Hydropsyche*

sparna and *H. slossonae* were also closely associated with each other. *Hydropsyche alternans* and *A. ladogensis* were associated with each other and with periphyton and pH. *Diplectrona modesta,* phytoplankton, velocity and temperature are not closely associated with other factors.

Table 4.11 Correlation matrix of the physical/chemical/nutrient variables with the species using standardized data.

Factor	*C. pettiti*	*H. betteni*	*H. sparna*	*H. slossonae*	*H. alternans*	*A. ladogensis*	*D. modesta*
pH	0.0436	-0.0481	0.0313	0.1579	-0.0166	0.1074	-0.2492
Conductivity	0.1812	-0.0094	-0.0783	-0.0378	-0.0892	-0.0662	-0.0368
Temperature	0.2399	0.2411	0.1089	-0.0671	0.0646	0.033	0.1521
Velocity	0.0544	0.1236	0.1975	0.116	-0.029	0.1252	0.0706
Periphyton	-0.005	-0.0018	-0.008	0.0328	-0.0107	0.0954	0.0314
Phytoplankton	-0.0541	-0.069	-0.0625	0.0301	-0.0275	-0.0353	0.0777
Zooplankton	0.1539	-0.0468	-0.041	0.0633	0.0146	-0.0328	-0.0296
C. pettiti	1	0.4489	-0.0778	0.154	0.0053	-0.0597	-0.0481
H. betteni	0.4489	1	-0.0253	-0.0919	-0.0352	-0.0523	-0.0237
H. sparna	-0.0778	-0.0253	1	0.0535	-0.0318	-0.0256	-0.0464
H. slossonae	0.154	-0.0919	0.0535	1	-0.0696	-0.0392	-0.0569
H. alternans	0.0053	-0.0352	-0.0318	-0.0696	1	-0.0296	-0.0241
A. ladogensis	-0.0597	-0.0523	-0.0256	-0.0392	-0.0296	1	-0.0265
D. modesta	-0.0481	-0.0237	-0.0464	-0.0569	-0.0241	-0.0265	1

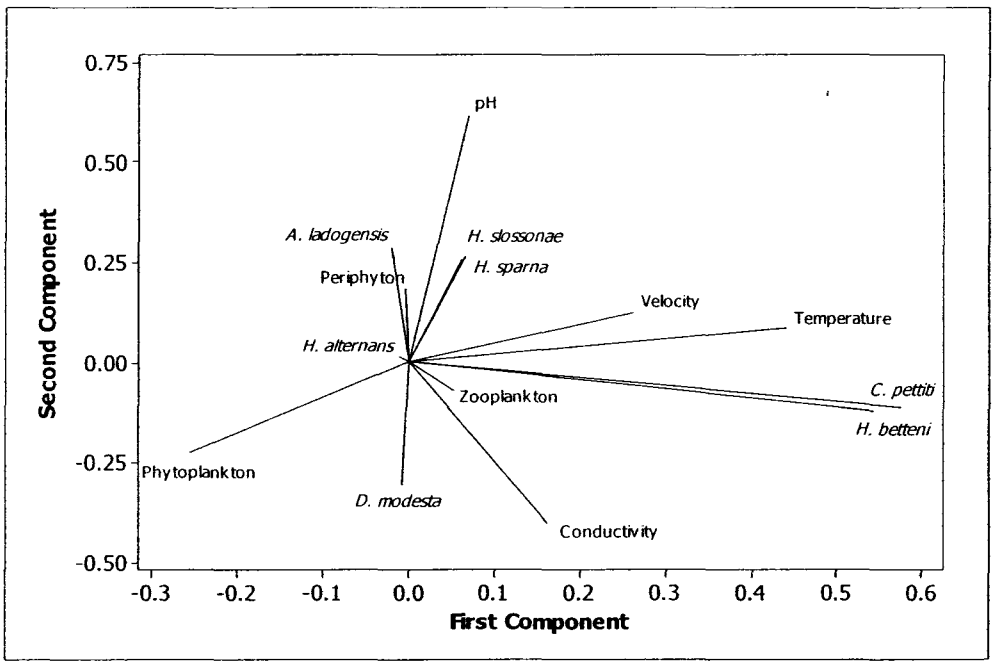

Figure 4.27 Loading plot of the first two components in a PCA of the physical, chemical nutrient variables and the species abundances.

Results from the canonical correlation between physical/chemical/nutrient variables and species abundances (Table 4.12) gave similar findings, with the first two factors explaining 70.9% of the total variance. The greatest canonical correlation is 0.3327 indicating that the covariance amongst these factors was low, which further emphasizes the weak influence the factors measured had on the abundances of species.

Table 4.12 Canonical correlation between the physical/chemical/nutrient variables and the species abundances, giving the correlations and the canonical correlations for the first and second set of variables and then for the relationship between these two sets.

Physical/Chemical/Nutrients						
	PCA1		PCA1 with Canonical1		Canonical1 with Canonical2	
Factor	axis1	axis2	axis1	axis2	axis1	axis2
pH	-0.5626	-0.0411	-0.3904	-0.1482	-0.1809	-0.0522
Conductivity	-0.3149	0.6878	-0.1591	0.6947	-0.0737	0.2448
Temperature	0.7517	0.468	0.718	0.3929	0.3327	0.1384
Velocity	0.3934	-0.4019	0.4541	-0.3379	0.2105	-0.1191
Periphyton	0.0901	-0.0902	0.0571	-0.1216	0.0265	-0.0428
Phytoplankton	-0.0275	0.0339	-0.0699	-0.0293	-0.0324	-0.0103
Zooplankton	-0.037	0.4097	-0.1285	0.4552	-0.0596	0.1604
Species Abundance						
	PCA2		PCA2 with Canonical2		Canonical2 with Canonical1	
Factor	axis1	axis2	axis1	axis2	axis1	axis2
C. pettiti	0.0878	0.9994	0.2493	0.7802	0.1155	0.2749
H. betteni	0.5623	-0.4065	0.5683	0.106	0.2633	0.0373
H. sparna	0.4338	-0.2066	0.365	-0.2888	0.1691	-0.1018
H. slossonae	-0.1025	-0.4421	-0.1768	-0.2455	-0.0819	-0.0865
H. alternans	0.2064	-0.101	0.1592	-0.0363	0.0738	-0.0128
A. ladogensis	0.1635	-0.2929	0.0976	-0.3067	0.0452	-0.1081
D. modesta	0.6792	0.0373	0.638	0.0437	0.2957	0.0154

4.3.2.1 Periphyton

Periphyton abundance (as expressed by chlorophyll-a concentration) clearly did not follow a log distribution with distance from outlets (Figure 4.28). However, large outliers at downstream stations affected mean abundances with most streams exhibiting large variation amongst stations (Figure 4.29). Abundances of periphyton followed a

linear distribution so a GLzM with an identity link and a gamma distribution was used. Periphyton abundances overall did not change with distance from outlets (p=0.1377). There was a significant interaction with landscape (p=0.0009), with abundances decreasing with distance in forested streams, although not significantly (p=0.1100), whereas downstream abundances significantly increased in barren streams (p=0.0016) (Table 4.13). Forested streams also had a higher intercept (Δ1146 mg/m^2) than barren streams (Table 4.13). There was not a significant interaction with time (p=0.7728), but there was with stream (p=0.0027 Table 4.13). Most streams did not exhibit significant changes in periphyton abundance although Broad Cove and Beaver Pond had highly negative slopes. Above Hatchet (p=0.0229) and Portugal Cove (p=0.0133) had highly positive slopes and were the only two streams that exhibited significant downstream changes (Table 4.13).

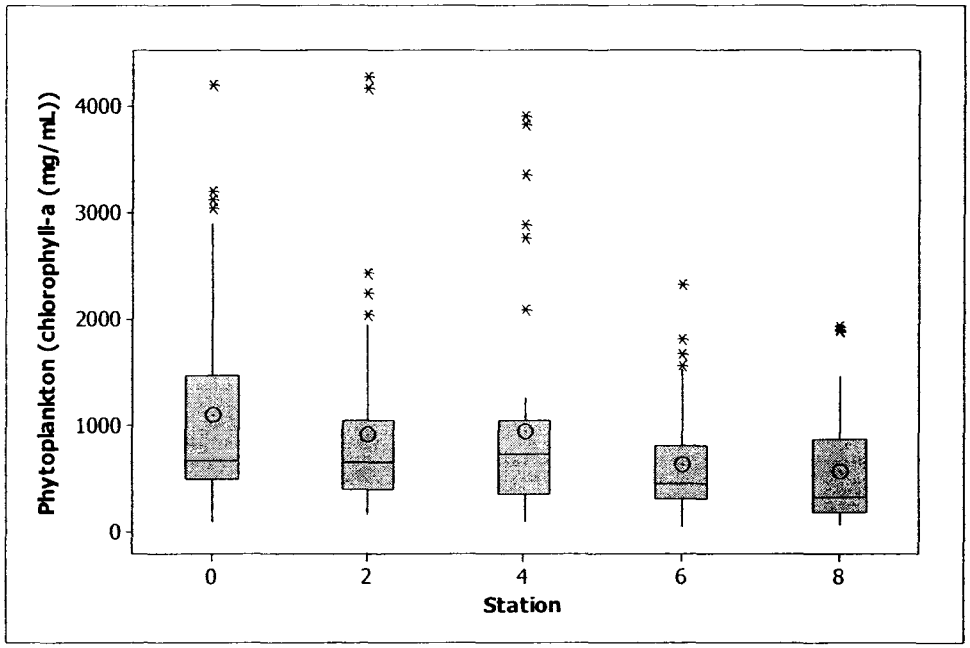

Figure 4.28 Boxplot of periphyton chlorophyll-a abundance from outlets downstream over all sampling times. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

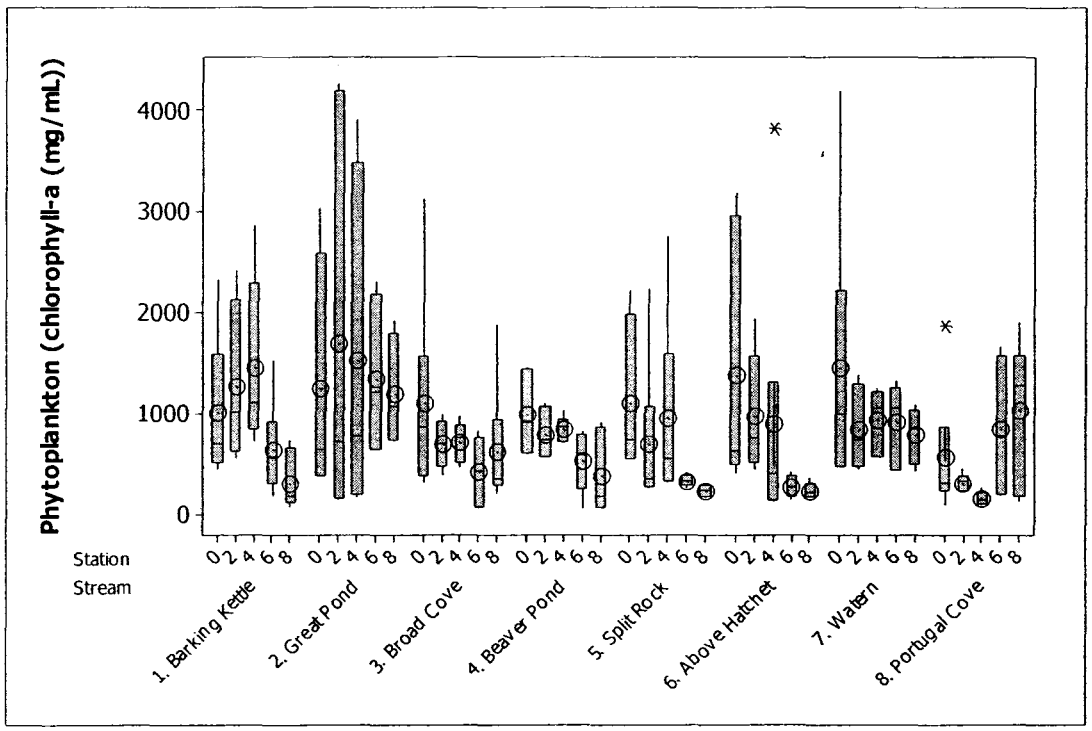

Figure 4.29 Boxplot of periphyton chlorophyll-a abundance by station for each stream over all sampling times. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

Table 4.13 Parameter estimates for the periphyton chlorophyll-a abundance with distance downstream (x); with distance downstream and landscape; with distance downstream and time; and with distance downstream and stream. The model uses an identity link with a gamma distribution (i=intercept, β=slope).

Variable	n	Parameter	Parameter Estimate	Likelihood Ratio 95% Confidence Limits	
x	336	i	6145.9280	5482.692	6902.706
		β	11.1002	-3.3385	28.0538
Landscape					
x*forested	156	i	6720.198	5758.3830	7883.612
		β	-15.1455	-31.3010	3.9032
x*barren	180	i	5574.4940	4728.4130	6588.181
		β	35.5061	12.4269	64.6969
Sampling time					
x*time1		i	na		
		β			
x*time2	111	i	5473.2300	4833.231	6216.406
		β	8.6350	-4.9567	24.8573
x*time3	117	i	5897.228	5267.069	6619.588
		β	6.1059	-6.3719	20.4553
x*time4	108	i	7042.1510	5171.818	9675.0570
		β	21.4571	-24.9287	94.9261
Stream					
x*Barking Kettle	45	i	3461.725	2822.636	4286.2130
		β	1.1168	-11.1842	16.7576
x*Great Pond	30	i	4126.291	3099.5920	5594.930
		β	22.0475	-4.1272	63.0232
x*Broad Cove	45	i	8484.5250	6242.483	11690.07
		β	-39.9213	-75.7738	6.5715
x*Beaver Pond	36	i	11019.99	8259.8590	15060.87
		β	-43.9190	-88.6647	11.0722
x*Split Rock	45	i	3799.775	2935.138	4963.167
		β	-2.6465	-19.1374	20.6796
x*Above Hatchet	45	i	5249.9280	3178.893	8624.696
		β	121.7663	9.2168	283.1750
x*Watern	45	i	5191.431	4020.625	6768.428
		β	6.1253	-18.6891	41.0922
x*Portugal Cove	45	i	7163.838	5298.138	9917.379
		β	61.6278	11.972	137.2739

4.3.2.2 Phytoplankton

Phytoplankton abundance (as expressed by chlorophyll-a concentration) generally declined slightly with increasing distance downstream (Figure 4.30). Phytoplankton abundances in Great Pond, Broad Cove, Beaver Pond, Split Rock, Above Hatchet and Watern generally followed a negative linear regression with distance downstream. Barking Kettle had a log normal distribution, and concentrations were elevated at stations four and five in Portugal Cove (Figure 4.31).

A GLzM with a log link and a gamma distribution was used to compare phytoplankton abundances to the derived model. Phytoplankton abundances did show a significant slight decline with increasing distance downstream ($p<0.0001$, Table 4.14). There were not significant differences with landscape ($p=0.7732$), but there was with time ($p=0.0001$) where time two showed a slight increase in phytoplankton abundances downstream and time three and four showed a decrease (Table 4.14). Abundances gently declined downstream in most streams, which was significant for Barking Kettle ($p=0.0084$), Beaver Pond ($p=0.0071$), Split Rock ($p=0.0001$) and Above Hatchet ($p<0.0001$) (Table 4.14). Only Portugal Cove had a slightly positive slope which was significant ($p=0.0447$, Table 4.14). Changes in phytoplankton abundance with increasing distance downstream clearly did not follow the derived model.

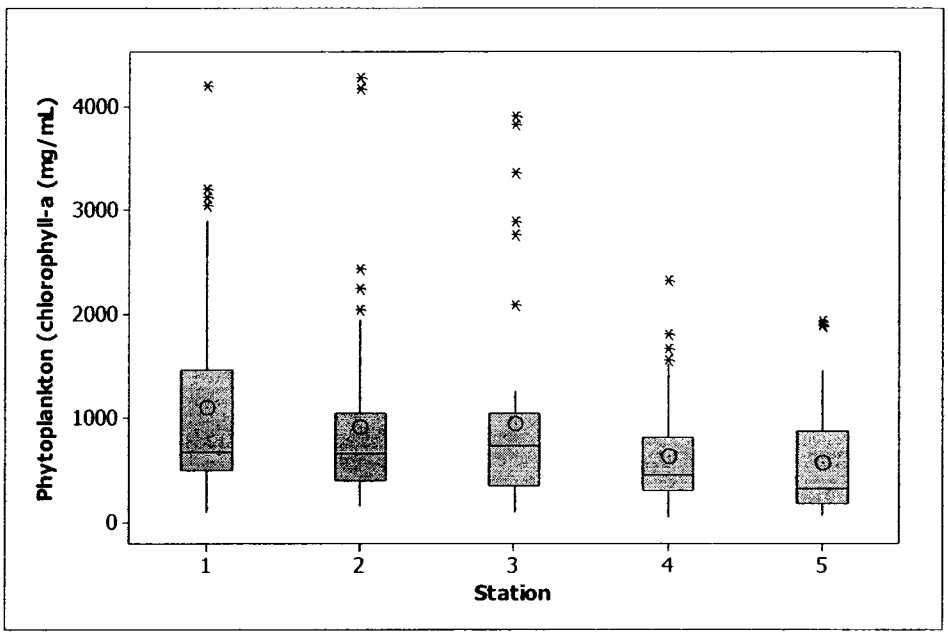

Figure 4.30 Boxplot of phytoplankton chlorophyll-a abundance from outlets downstream over all sampling times. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

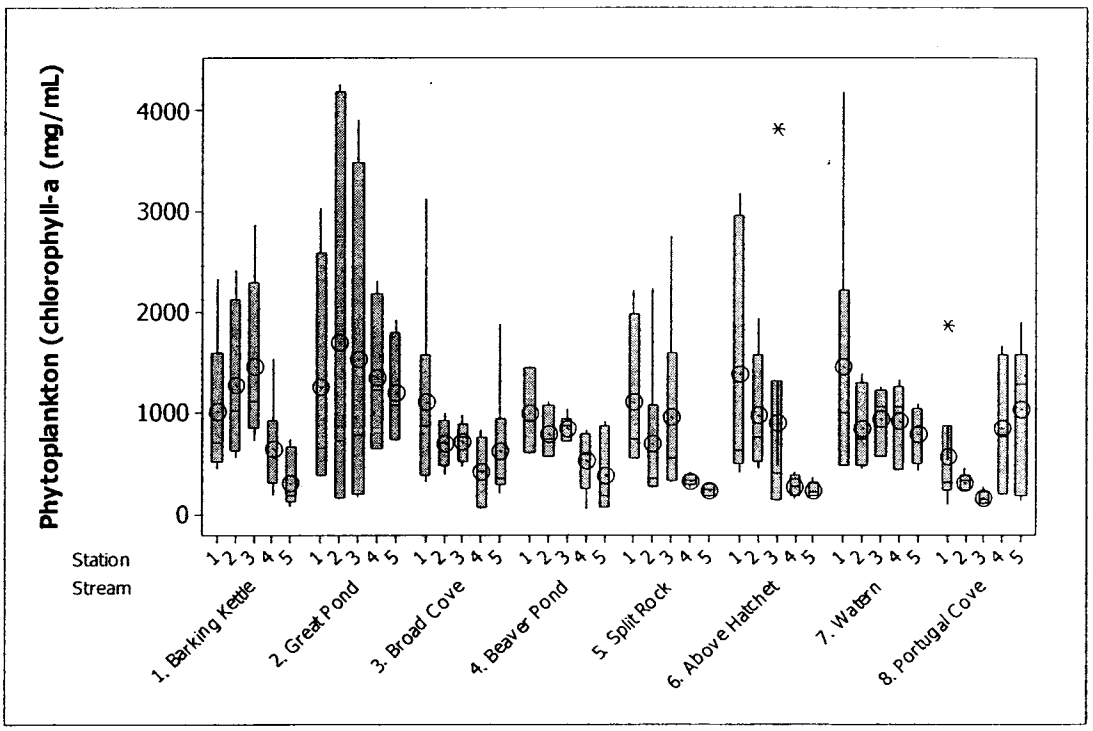

Figure 4.31 Boxplot of phytoplankton chlorophyll-a abundance by station for each stream over all sampling times. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

Table 4.14 Parameter estimates for the phytoplankton chlorophyll-a abundance with distance downstream (x); with distance downstream and landscape; with distance downstream and time; and with distance downstream and stream. The model uses a log link with a gamma distribution (i=intercept, β=slope).

Variable	n	Parameter	Parameter Estimate	Likelihood Ratio 95% Confidence		Fits model?
x	236	i	7.0390	6.8666	7.2194	no
		β	-0.0827	-0.1192	-0.0462	
Landscape						
x*forested	116	i	7.1563	6.9111	7.4171	no
		β	-0.0874	-0.1408	-0.0337	
x*barren	120	i	6.9085	6.6728	7.1602	no
		β	-0.0767	-0.126	-0.0275	
Sampling time						
x*time1		i	na			
		β				
x*time2	76	i	6.0580	5.8638	6.2628	no
		β	0.0308	-0.0106	0.0724	
x*time3	80	i	6.9350	6.7298	7.1513	no
		β	-0.0902	-0.1335	-0.0469	
x*time4	80	i	7.6332	7.2891	8.0098	no
		β	-0.1333	-0.2079	-0.0588	
Stream						
x*Barking Kettle	30	i	7.3941	6.9129	7.9293	no
		β	-0.1504	-0.2593	-0.0414	
x*Great Pond	26	i	7.3166	6.7586	7.9608	no
		β	-0.0139	-0.1487	0.1254	
x*Broad Cove	30	i	6.8670	6.4757	7.3044	no
		β	-0.0794	-0.1025	0.0038	
x*Beaver Pond	30	i	6.9827	6.6066	7.3978	no
		β	-0.1155	-0.1975	-0.0337	
x*Split Rock	30	i	7.1462	6.7442	7.5916	no
		β	-0.1893	-0.2778	-0.1008	
x*Above Hatchet	30	i	7.3782	6.9195	7.8940	no
		β	-0.2341	-0.3348	-0.1329	
x*Watern	30	i	7.1242	6.8195	7.4565	no
		β	-0.0589	-0.1234	0.0053	
x*Portugal Cove	30	i	5.9344	5.4747	6.4669	no
		β	0.0996	0.0025	0.1959	

4.3.2.3 Zooplankton

Mean abundances of zooplankton for all streams combined dropped off sharply between station one and two and remained low further downstream, with peaks at station one observed in four of the eight streams (Figure 4.32, Figure 4.33). Barking Kettle and Broad Cove had more than double the amount of zooplankton observed at Beaver Pond and Watern outlets (Figure 4.33), whereas the remaining four streams had low abundances throughout.

Changes in zooplankton abundance for all streams combined with distance downstream was highly significant ($p<0.0001$) but the parameter estimates did not fit the derived decay model (Table 4.15). There was a significant difference between landscape type ($p=0.0079$), with forested landscapes having higher abundances at outlets (Δ47/50L) and much steeper declines with increasing distance downstream (β = -0.3707) than in barren landscapes (β = -0.1804, Table 4.15). There was not a significant change in the downstream abundance among sampling times ($p=0.4992$), with all sampling times having significant decreases in downstream abundances. There were significant differences amongst streams ($p=0.0006$, Table 4.15). All streams had a negative slope, with the exception of Great Pond which did not show changes in abundance as it did not follow a negative power function. Only the four streams with elevated outlet abundances decreased significantly below outlets, with one of these streams, Broad Cove, fitting the derived model (Table 4.15).

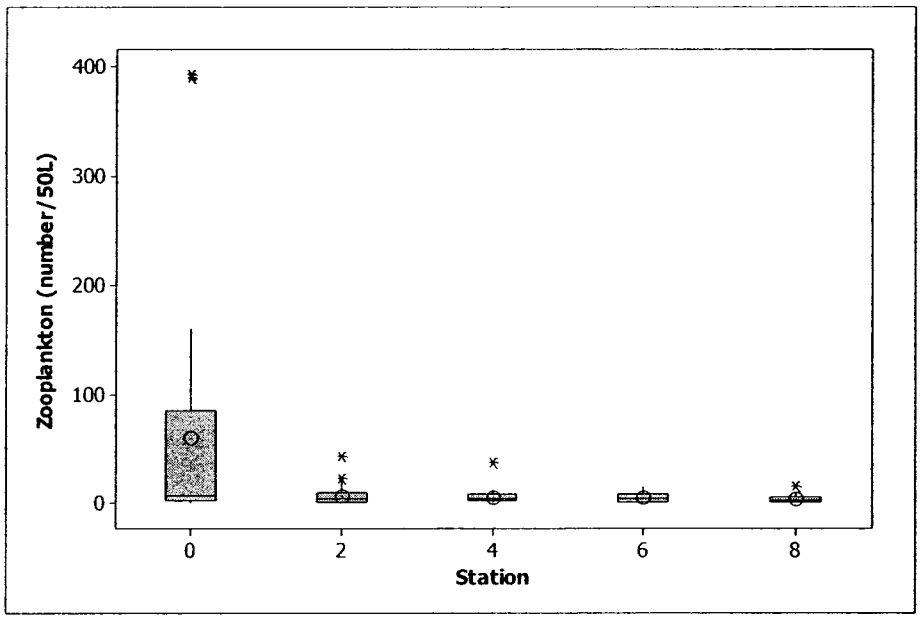

Figure 4.32 Boxplot of zooplankton abundance from outlets downstream. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

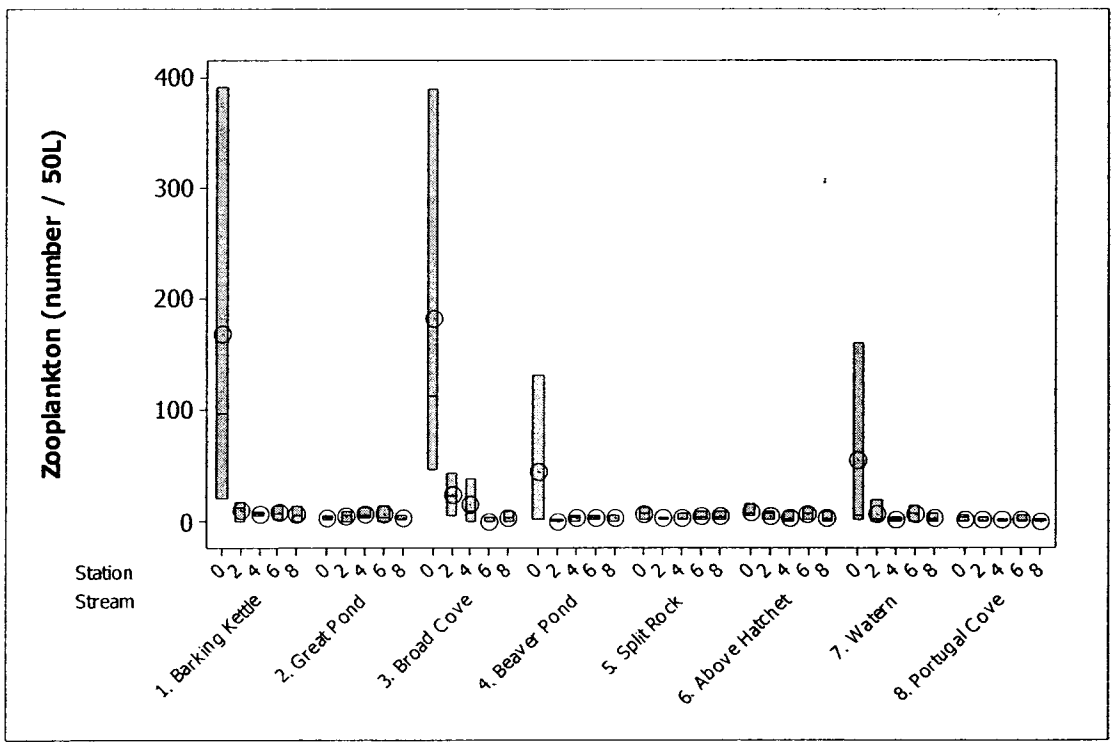

Figure 4.33 Boxplot of zooplankton abundance by station for each stream. Asterisks indicate outliers, open circles the mean and boxes are the interquartile range with the line indicating the median.

Table 4.15 Parameter estimates for the zooplankton abundance with distance downstream (x); with distance downstream and landscape; with distance downstream and time; and with distance downstream and stream (i=intercept, β=slope).

Variable	n	Parameter	Parameter Estimate	Likelihood Ratio 95% Confidence		Fits model?
x	120	i	3.5838	3.2374	3.9484	no
		β	-0.3152	-0.3886	-0.2423	
Landscape						
x*forested	60	i	4.0811	3.6142	4.6212	no
		β	-0.3707	-0.4715	-0.2706	
x*barren	60	i	2.5433	2.0901	3.0556	no
		β	-0.1804	-0.2789	-0.0830	
Sampling time						
x*time1	119	i	na			
		β				
x*time2	120	i	3.4737	2.8252	4.2728	no
		β	-0.3646	-0.5138	-0.2194	
x*time3	120	i	3.3331	2.7621	4.0096	no
		β	-0.2581	-0.3849	-0.1328	
x*time4	120	i	3.8630	3.3344	4.4875	no
		β	-0.3271	-0.4414	-0.2135	
Stream						
x*Barking Kettle	15	i	4.4996	3.7336	5.4931	no
		β	-0.3779	-0.5508	-0.2063	
x*Great Pond	15	i	1.6987	1.0660	2.3673	no
		β	0	-0.1358	0.1355	
x*Broad Cove	15	i	4.7631	3.8635	5.9649	yes
		β	-0.4821	-0.6927	-0.2693	
x*Beaver Pond	15	i	3.1614	2.2106	4.4792	no
		β	-0.2947	-0.5207	-0.0733	
x*Split Rock	15	i	1.7269	1.1490	2.3342	no
		β	-0.0269	-0.1469	0.0932	
x*Above Hatchet	15	i	2.1349	1.5276	2.8073	no
		β	-0.0695	-0.2031	0.0625	
x*Watern	15	i	3.5112	2.5342	4.8792	no
		β	-0.3155	-0.5538	-0.0852	
x*Portugal Cove	15	i	0.9370	-0.0226	2.0346	no
		β	-0.0755	-0.2926	0.1366	

4.3.3 Comparisons of trends among periphyton, phytoplankton, and zooplankton abundance with hydropsychid abundance

General trends in the longitudinal distribution of periphyton, phytoplankton and zooplankton were compared to hydropsychid abundance. This was possible for hydropsychids with higher abundances using the appropriate link function: outlet species (log link); *C. pettiti* (log link); *H. betteni* (log link); downstream species (identity link); *H. sparna* (identity link); and *H. slossonae* (identity link).

Periphyton abundance remained relatively constant with distance downstream and so did not reflect the overall distribution of outlet species, *C. pettiti* or *H. betteni*. Linear downstream trends in periphyton abundance were found to be similar to that of downstream species, *H. sparna* and *H. slossonae,* in all instances (overall, with sampling time and with individual streams) with the exception of Portugal Cove where periphyton abundances increased downstream and hydropsychid abundances decreased.

Phytoplankton abundances did not follow an overall similar trend to abundances of outlet species, *C. pettiti* or *H. betteni*. There were no similar trends in the distribution of outlet species and phytoplankton, nor were there for *C. pettiti* among individual streams. For *H. betteni* there were only similar trends in two streams, Great Pond ($p=0.2567$) and Split Rock ($p=0.7674$). Downstream species abundance showed a similar trend to phytoplankton abundance in the second sampling time ($p=0.7156$) over all streams and in two streams when considered separately (Split Rock ($p=0.0514$) and Watern ($p=0.7940$)). Trends in phytoplankton abundance were only compared with *H. slossonae* in Above Hatchet, Watern and Portugal Cove because the species declined

from the outlet in these streams. Abundances of phytoplankton and *H. slossonae* showed similar trends in Watern (p=0.0856) and Portugal Cove (p=0.1785). For *H. sparna* trends were similar in Barking Kettle (p=0.1139), Split Rock (p=0.6110) and Watern (p=0.0673).

Outlet species abundances declined at a faster rate than zooplankton with only Great Pond (p=0.1089) and Split Rock (p=0.4502) showing similar trends. Overall the longitudinal distribution of zooplankton was significantly different from that of *C. pettiti* which declined at a faster rate. Similar trends were seen with *C. pettiti* and zooplankton abundances in only two streams, Beaver Pond (p=0.0887) and Great Pond (p=0.0770). There were strong similar trends between zooplankton and *H. betteni* abundances, with overall abundances showing a similar rate of decline (p=0.3313). This held true in forested (p=0.7682) and barren (p=0.0730) landscapes (Figure 4.34) and over all sampling times. Trends were similar in only two streams when considered individually, Great Pond (p=0.5349) and Split Rock (p=0.4564). Trends generally differed among abundances of downstream species and zooplankton with the exception of one stream, Watern (p=0.5714). Trends in zooplankton abundance were compared to *H. slossonae* in Above Hatchet, Portugal Cove and Watern as the abundance of this species generally declined from the outlet, with Watern (p=0.1256) exhibiting a similar trend in slopes. There were no similar trends in zooplankton abundance and *H. sparna* or downstream species abundance.

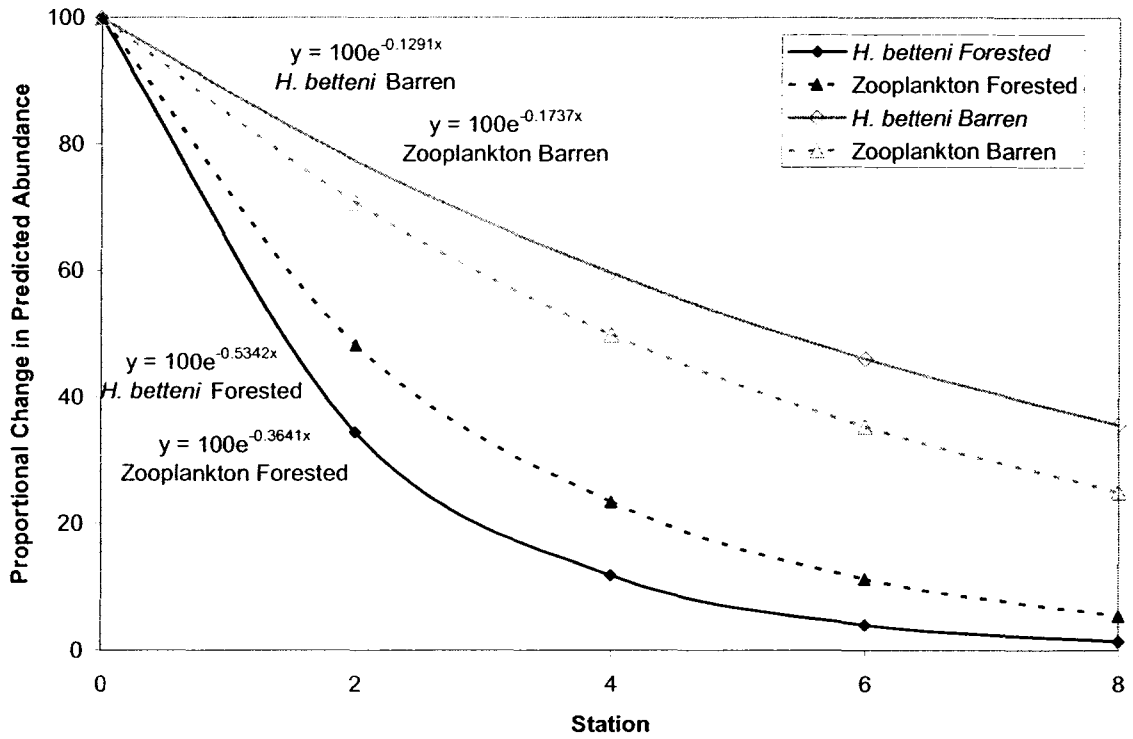

Figure 4.34 Change in proportion of *H. betteni* and zooplankton abundance from successive stations, separated into forested and barren landscapes.

Total hydropsychid abundances (sums of all species of hydropsychids at each station) followed a decay model below outlets with an overall gentler slope than outlet species (β= -0.2008) and were compared to abundances of phytoplankton and zooplankton. Trends in total hydropsychid abundance were similar to phytoplankton abundance by time, by landscape and by stream. Overall the longitudinal distribution of zooplankton was significantly different from total hydropsychid abundance which declined at a slower rate, but there was a similar trend in barren streams (p=0.4159) but not in forested streams (p=0.0469) (Figure 4.35). Trends were similar over all sampling

times and in five individual streams: Barking Kettle (p=0.3213), Great Pond (p=0.8961), Broad Cove (p=0.6965), Split Rock (p=0.7753) and Above Hatchet (p=0.6028).

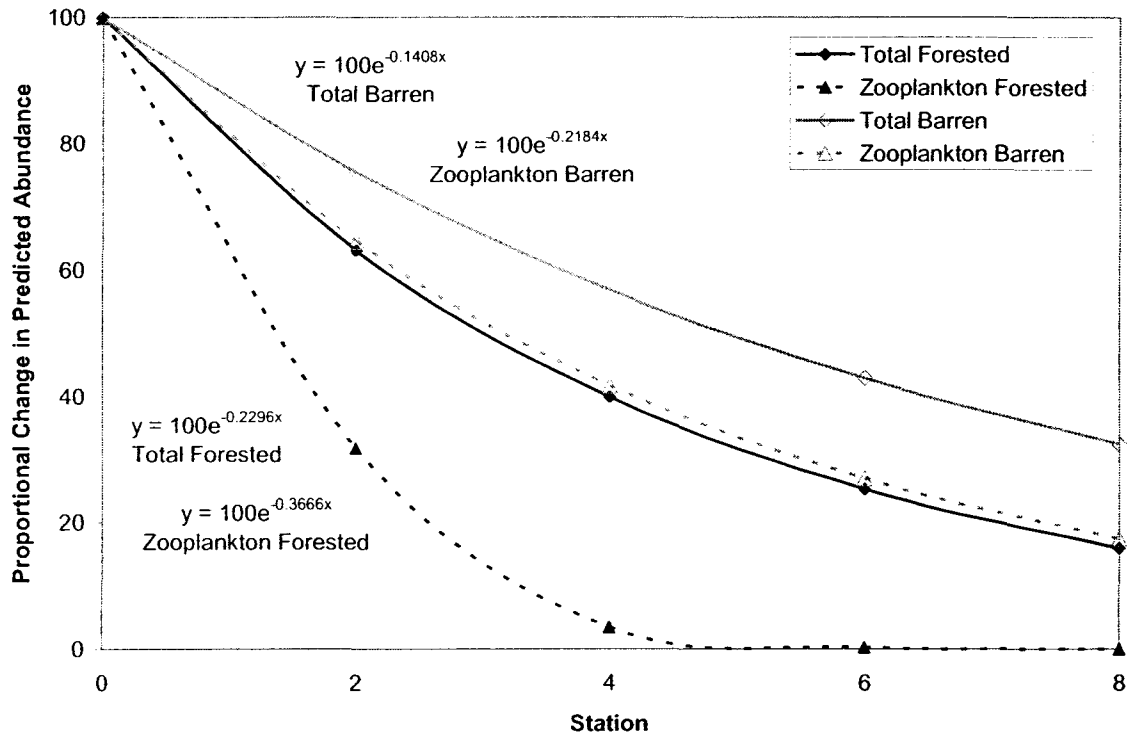

Figure 4.35 Change in proportion of total hydropsychids and zooplankton abundance from successive stations, separated into forested and barren landscapes.

4.3.4 Is the abundance of *H. sparna* at Station 4 correlated with that of *C. pettiti* at Station 1?

Abundances of *H. sparna* were highest at Station 4 and declined slightly downstream (Figure 4.23 & Figure 4.24), while that of *C. pettiti* decreases from the outlet and reaches a low point at Station 4 (Figure 4.10 & Figure 4.11). The abundance of *H. sparna* at Station 4 was regressed against that of *C. pettiti* at the outlet for each stream to determine if there was a relationship. A regression using the normal distribution was not

an appropriate model for the data; instead a negative binomial distribution with a log link was used. Most streams did not show a significant correlation ($p>0.05$) with the exception of Watern ($p=0.0004$, $R^2=0.5736$, Figure 4.36).

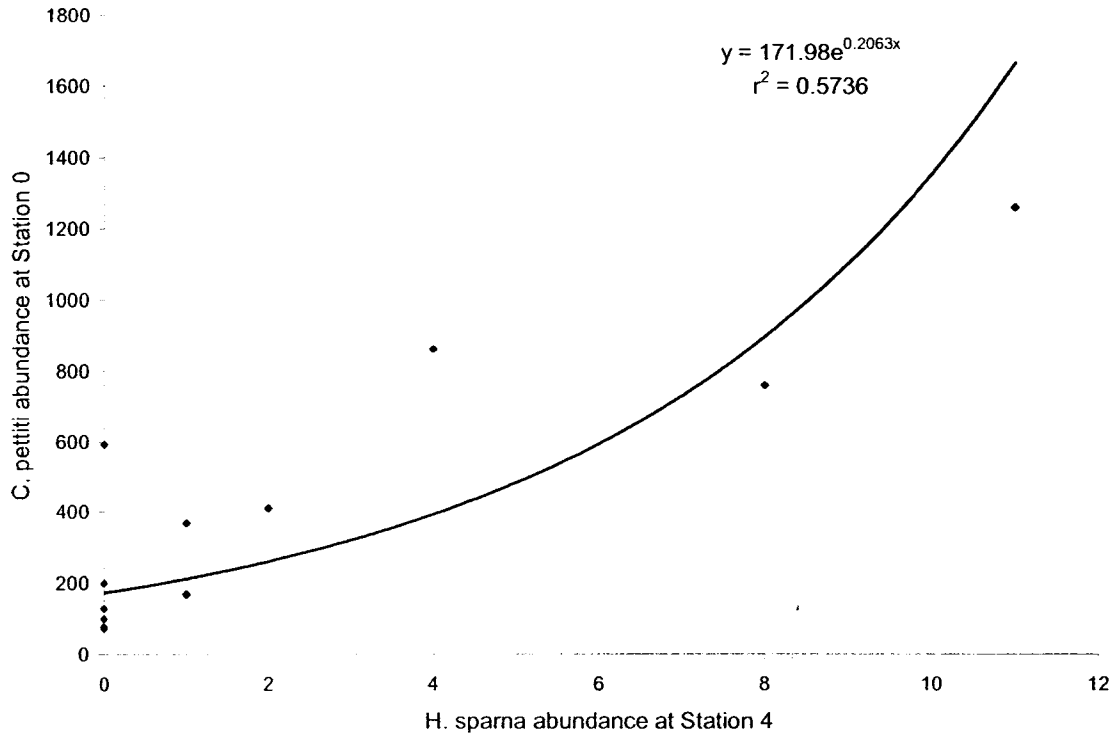

Figure 4.36 A regression of the abundance of *H. sparna* at Station 4 versus *C. pettiti* at Station 0 for Watern.

4.3.5 Temperature probes

Water temperatures were significantly elevated at outlets compared to downstream sites ($p<0.0001$) (Figure 4.37; Table 4.16) and streams thought to be groundwater-fed were significantly cooler ($p<0.0001$) (Figure 4.37; Table 4.16). Outlets were 0.68°C warmer on average than downstream sites from June 1 to October 31 2003.

Water temperatures were significantly elevated in forested compared to barren landscapes ($p<0.0001$) (Figure 4.38; Table 4.16) and streams thought to be groundwater-fed were significantly cooler ($p<0.0001$) (Figure 4.38; Table 4.16). Forested streams were 0.92°C warmer on average than barren streams from June 1 to October 31 2003.

Table 4.16 One-way ANOVAs comparing water temperatures by location, landscape and groundwater-fed.

One-way ANOVA: outlet, downstream, groundwater-fed

```
Source      DF       SS      MS       F      P
Factor       2    25591   12795  757.39  0.000
Error    65474  1106114      17
Total    65476  1131705
S = 4.110   R-Sq = 2.26%   R-Sq(adj) = 2.26%
                    Individual 95% CIs For Mean Based on Pooled StDev of 4.110
Level               N    Mean  StDev  -----+---------+---------+---------+----
outlet          21805  15.533  4.323                                      (*)
downstream      28984  14.856  4.057                           (*)
groundwater-fed 14688  13.826  3.884  (-*)
                                      -----+---------+---------+---------+----
                                        14.00     14.50     15.00     15.50
```

One-way ANOVA: forested, barren, groundwater-fed

```
Source      DF       SS      MS       F      P
Factor       2    31052   15526  895.87  0.000
Error    69146  1198348      17
Total    69148  1229400
S = 4.163   R-Sq = 2.53%   R-Sq(adj) = 2.52%
                    Individual 95% CIs For Mean Based on Pooled StDev of 4.163
Level               N    Mean  StDev  -----+---------+---------+---------+----
forested        29376  15.552  4.217                                      (*)
barren          25085  14.635  4.256                        (*)
groundwater-fed 14688  13.826  3.884  (-*)
                                      -----+---------+---------+---------+----
                                        14.00     14.50     15.00     15.50
```

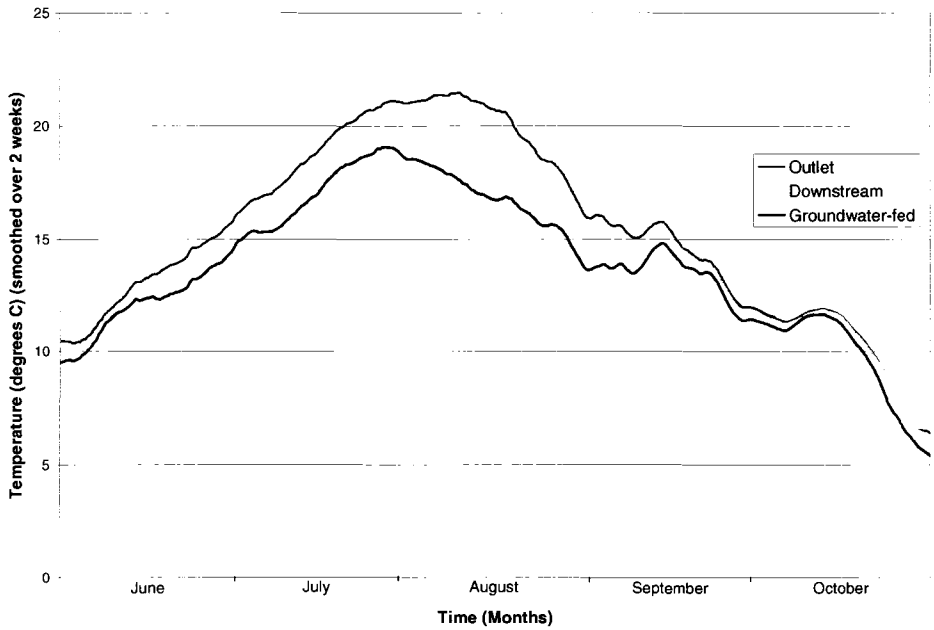

Figure 4.37 Stream temperatures at outlets, downstream sites and in presumably groundwater-fed streams versus time in months, with temperature smoothed using a two week moving average.

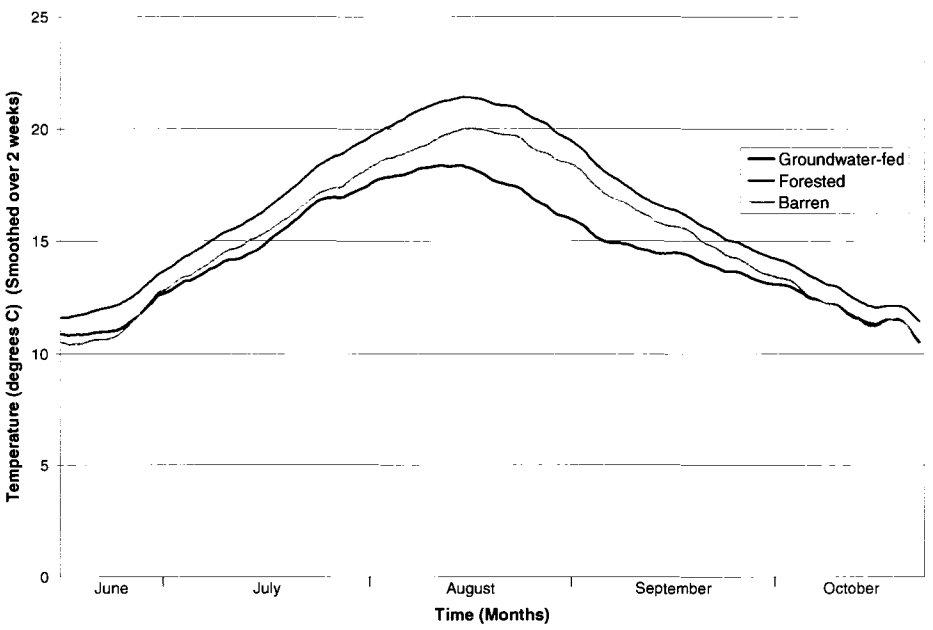

Figure 4.38 Stream temperatures in forested and barren landscapes and in presumably groundwater-fed streams versus time in months, with temperature smoothed using a two week moving average.

4.4 Discussion

4.4.1 Use of rock bags in the eight chosen streams

General patterns in the eight streams studied here agree with the broader survey presented in Chapter 2. Abundances of outlet species (*C. pettiti, H. betteni* and *H. alternans*) declined quickly downstream of lakes as anticipated. Other hydropsychid taxa either changed little in abundance or had an increased abundance downstream. Generally, none of the downstream species (*H. sparna, H. slossonae, A. ladogensis* and *D. modesta*) reached the abundances of the outlet species. Some species (*H. alternans, A. ladogensis* and *D. modesta*) had a patchy distribution both within and among streams. *Hydropsyche alternans* occurred in two streams but was abundant in only one. *Arctopsyche ladogensis* occurred mainly downstream in larger streams, whereas *D. modesta* occurred in three smaller streams (Barking Kettle, Great Pond and Broad Cove) in low abundances except at the sixth station in Barking Kettle during late July to late August. Therefore the communities in the eight streams provided a good profile of Newfoundland Hydropsychidae to use to comparative model the influence of lakes, landscapes and stream size here.

Rock bag samplers used here were very effective sampling units for comparative studies of hydropsychid species among and within streams. For example, even taxa that were known to have patchy distributions (*H. alternans, A. ladogensis* and *D. modesta*) were all collected in significant numbers in sites where they occurred. This was partly because rock bags were designed to be ideal hydropsychid habitat based on previous studies of larval hydropsychid colonization requirements (Cardinale et al. 2001; Elser 1999; MacKay 1992). In addition, all rock bags were placed in riffle areas with similar

flow patterns to reduce variance between samples. Colonization of sites by hydropsychids is related to substrate size and stability (Barber & Kevern 1973; Benke et al. 1984; Malmqvist & Otto 1987), water velocity and flow pattern (Becker 1987; Edington 1968; Fuller & MacKay 1980b; Osborne & Herricks 1987; Wallace 1975a), moss cover (Haefner & Wallace 1981; Minshall 1984) and presence of invertebrate predators (Michael & Culver 1987).

Flow rates and other factors influence hydropsychid density. Under low flow conditions larvae attacked neighbouring retreats more frequently (Matczak & MacKay 1990), possibly because there were lower rates of particle capture by their nets (Georgian & Thorp 1992). Also under low flow rates, larvae were more likely to abandon their retreats in search of more suitable conditions and were then more prone to be caught in existing nets and devoured (Philipson 1969). Under high flow laboratory conditions, larvae constructed more nets as they spent less time searching for appropriate sites (Becker 1987; Edington 1968; Philipson 1969; Philipson & Moorhouse 1974). Positioning within an aggregation of individuals influenced larval growth, with smaller individuals found in the rear (downstream) of aggregations where flow had decreased (Englund 1991). Philipson (1969) demonstrated the effect of optimal flow rates and temperature ranges on increased net construction and Fairchild & Holomuzki (2002) recognized the importance of substrate size and stability and seston quantity on hydropsychid abundances. These examples emphasize the importance of intraspecific competition and environmental variables on hydropsychid colonization and abundance.

Hydropsychids will only colonize appropriate sites, which influences their distribution and abundance at larger spatial scales (i.e. multiple streams) (Kerans et al. 2000). Colonization can also be density dependent, so there is an upper limit to the density a site can support. Englund (1993) found hydropsychid colonization to be high at highly populated sites. In the present study, all organisms were removed from rock bags at each sampling time, leaving a substrate clean of conspecifics and potential predators which may have engendered rapid recolonization. The fact that rock bag samplers were colonized with up to several hundred larvae in a few weeks demonstrated considerable movement of larvae within a stream, with this occurring through all the sampling times. This may be caused by a combination of population density and drifting as Kerans et al. (2000) found spring populations of *H. slossonae* to infrequently disperse via drifting, but that the drifting rate was strongly density dependent. Overall the rock bags matched these requirements as the bag was flexible and fitted into pockets between the exiting large substrates reducing movement in a spate the numerous small stones provided a large surface and interstial space area while the bag held the small stones within it stable. They also permitted sampling sites such as boulder fields which were not feasible with a surber.

4.4.2 Patterns of outlet species abundance

From Chapter 2 it was known that *C. pettiti, H. betteni* and *H. alternans* occurred most frequently near outlets, often in high abundances. This chapter explored patterns below outlets and so the approach of forming two groups of species, outlet and downstream, allowed consideration of general trends. Using all streams and all sampling

times, the confidence limits of the rate of decrease of outlet species did fit the slope of the derived model. When forested streams were considered separately outlet species declined faster than the derived model because of high outlet abundances, but rates of decline in barren streams were very similar to the derived model. Confidence limits of the slope in three of the four sampling times also fit the derived model and so the modeling was robust over time. Modeling was not robust in individual streams, with only two of the eight having similar slopes to the derived model.

Higher abundances of outlet species were generally at outlets and the second station, possibly because of adult behaviour. Gravid adult females tend to fly upstream to oviposit. When stream conditions end at lake outlets, females lay their eggs and so colonization rates at outlets are high (Roos 1957). Hydropsychids drift (MacKay 1992) and so outlet species may drift downstream after hatching to avoid competition with other filter feeders or because of environmental conditions. *Hydropsyche betteni* extended further downstream than *C. pettiti*, and so may have a wider tolerance range to changes in temperature and nutrient concentrations.

4.4.2.1 *Cheumatopsyche pettiti*

Abundances of *C. pettiti* declined more rapidly than all other outlet species and were higher in forested than barren streams, agreeing with the general survey data in Chapter 2. Although the rate of decline using all streams and sampling times did not follow the derived model, it fit in four (Great Pond, Beaver Pond, Watern, Portugal Cove) of the seven streams where *C. pettiti* occurred and included both forested and barren landscapes. Rates of decline were greater for the remaining three stream systems (Above

Hatchet, Broad Cove, Barking Kettle) indicating that *C. pettiti* was chiefly restricted to outlets followed by a steep decline. These small streams had pools immediately below their outlets which may have reduced downstream movement of the population. Mackay & Waters (1986) also found the distribution of *C. pettiti* to be limited to short distances below outlets. Elsewhere in North America *C. pettiti* was not restricted to outlets (Kondratieff et al. 1997; MacKay 1986; MacKay & Waters 1986; Sanchez & Hendricks 1997), indicating that perhaps in this study food abundance and/or quality as wells as other physiochemical variables were unsuitable downstream.

The pattern of temporal scale variation observed would differ from the actual pattern for *C pettiti* as the early instars were not identified to the species. Rates of decline were higher than the derived model with the exception of the third sampling period where there was less change in abundances of *C. pettiti* from outlets to the second station. This was likely because of the development of early instars into identifiable larvae and because high densities at outlets of new cohorts resulted in downstream drift. The second and third sampling times had high numbers of unidentifiable early instars at outlets. The subsequent decline of these early instars and dramatic increase in the number of *C. pettiti* larvae suggest emergence and oviposition had occurred from July to August. This was similar to other studies of *C. pettiti* in North America where it was found to have a long emergence time from May to October (MacKay 1986; Sanchez & Hendricks 1997).

Consideration of hydropsychid community composition showed this species occurred in high abundances at outlets and was mostly restricted to outlets. There was no significant correlation between *C. pettiti* and zooplankton abundance, however both were

elevated at outlets and so *C. pettiti* may take advantage of this food source since *Cheumatopsyche* have been reported to consume animal material (Coffman et al. 1971). Multiple linear regressions (Table 4.6) showed that YSI readings of temperature and conductivity significantly correlated with *C. pettiti* abundance. However, the associated r^2 values were low (0.1216 for temperature, 0.1830 for conductivity) indicating a weak influence of these factors.

4.4.2.2 *Hydropsyche betteni*

Hydropsyche betteni was the second most abundant species at outlets, but its abundance declined less rapidly than *C. pettiti*. Its rate of decline over all streams and times did not fit within the 95% confidence limits of the derived model (Figure 4.17), but the slope was much closer to it than that of *C. pettiti* overall (Figure 4.12). Data from forested streams did fall within the 95% confidence limits of the derived model (Figure 4.18), especially in Barking Kettle (Figure 4.19). In barren landscapes outlet abundances were low as was the rate of decline (Figure 4.19), which did not fit the model, but this was skewed by one stream (Split Rock) where *H. betteni* occurred throughout the sampled reach. More barren streams need to be sampled to draw a firm conclusion. The rarity of this in barren streams sampled here agrees with the broad survey, Chapter 2, where greater abundances were found at forested versus barren outlets. This does not appear to relate to nutrient availability as very weak negative correlations were seen between its abundance and periphyton, phytoplankton and zooplankton abundance (<7%, Table 4.7). Its abundance did significantly correlate with all of the physiochemical

variables (Table 4.6), but correlations were weak making the influence of these variables hard to interpret.

The model was nevertheless quite robust in three of the four sampling times. The exception was the third sampling, but even then there was no great discrepancy between the derived model slope and the 95% confidence limits. The change in *H. betteni* abundance from outlets to station two was greater during the first and second sampling times causing slopes of these regression lines to be steeper than those of sampling times three and four. However, like with *C. pettiti,* young, unidentifiable larvae at outlets may have included *H. betteni*. A decline in *H. betteni* abundance from the first to the second sampling time suggests adult emergence and oviposition was occurring, thus the increase seen in the third and fourth sampling times reflected when the small larvae had reached a size that could be identified. Downstream populations likely resulted from downstream drift, which is thought to be a mechanism for avoiding predation and competition (Holomuzki et al. 1999; Kerans 1996). The slower downstream decline of *H. betteni* compared to *C. pettiti* could have been caused by a higher drift rate of *H. betteni.*

Distributions of *H. betteni* here agreed with those of other studies where this species was found throughout streams but abundances were greatest below outlets (Fairchild & Holomuzki 2002; Genge 1985; MacKay 1979). Mackay (1979) also found occurrences of *H. betteni* to be quite variable between streams, with only 10 specimens found in the Credit River which neighbours the Humber River where this species was abundant. Mackay (1979) also found populations below outlets to be bivoltine, whereas upstream ones were univoltine. In this study, observations at Barking Kettle outlet

suggested that *H. betteni* was bivoltine, with overlapping cohorts which would account for the high abundances found there. Temperatures at this small outlet were elevated which can affect both voltism (MacKay 1979; MacKay 1984), and abundance (Fairchild & Holomuzki 2002).

In Great Pond and Split Rock *H. betteni* had a similar rate of decline as phytoplankton, which agreed with findings by Fairchild & Holomuzki (2002) in a Michigan stream. Overall the rate of decline of this species was similar to that of zooplankton, indicating that this may also be an important food resource. Outlets were also significantly warmer, an association also supported by Fairchild & Holomuzki (2002), and so a combination of phytoplankton and zooplankton abundances and water temperature was influencing the distribution of *H. betteni.*

4.4.2.3 *Hydropsyche alternans*

Hydropsyche alternans only occurred in the largest two streams and abundances were highest near outlets, particularly in the forested stream. This agrees with the broad survey of Chapter 2 where it primarily occurred at outlets, had higher abundances in forested landscapes and had a lower frequency of occurrence (~35% of the sites sampled) than the other two outlet species. *Hydropsyche alternans* has a very broad North American distribution, from wave-washed lakeshores to large rivers (Milne 1943; Schefter & Wiggins 1986) but little is known of its biology (Schuster & Etinier 1978). Here it generally occupied the outlet niche when abundances of *C. pettiti* and *H. betteni* were low.

4.4.3 Downstream species

Collective abundances of downstream species were highly variable both within and among streams. Two species *A. ladogensis* and *D. modesta* made only a minor contribution to overall stream abundances as they only had high abundances at one site each in separate streams. Overall the downstream community was much less distinct than the outlet community in terms of its distribution throughout a stream. This agrees with the broad survey in Chapter 2 where there were no significant differences in the occurrence or abundance of these four species with location or landscape and so this finding appears to generally apply to Newfoundland streams. Therefore the downstream community is generally dominated by two widely distributed species, *H. sparna* and *H. slossonae*.

4.4.3.1 *Hydropsyche sparna*

Abundances of *Hydropsyche sparna* were quite variable, although a straight line model generally fit its distribution in all but one stream, Beaver Pond. There its distribution followed a log normal curve but the agreement of this curve to the data could not be tested with available software. Abundances generally did not differ by station or by landscape which agrees with the broad survey of Chapter 2. This agrees with Genge (1985) who found *H. sparna* throughout a small Newfoundland stream. Elsewhere in North America *H. sparna* occurs throughout streams (Fairchild & Holomuzki 2002; MacKay 1979; Rutherford & MacKay 1986), although it rarely occurred within 20 metres of outlets (MacKay 1979). This may be because it does not depend on fast currents for net spinning (Fuller & MacKay 1980b) and/or has a diet high in detritus (Fuller & MacKay 1980a). This species is thus well suited to a large array of stream

habitats and is a generalist, able to tolerate a wide range of conditions compared to other hydropsychids (Fairchild & Holomuzki 2002).

During the third and fourth sampling times an increased abundances of young, unidentifiable larvae from stations two to eight were present. This corresponded with increased abundances of *H. sparna,* suggesting that early instars were developing into identifiable *H. sparna* larvae. This would cause higher abundances downstream, resulting in the negative slopes seen during this time period for *H. sparna.*

Hydropsyche sparna became more abundant when the abundance of outlet species declined. This indicates that this species was able to colonize sites with a low suitability to other hydropsychids and demonstrated its ability to utilize a range of resources (Table 1.3; section 1.7.8) (Richardson & MacKay 1991). Lower abundances at outlets indicated that outlet conditions were less optimal for *H. sparna* (MacKay 1979). In this study, longitudinal abundances of *H. sparna* and periphyton had similar distributions indicating it may be responding to primary productivity, but the weak direct correlation between the two (Table 4.11) indicates that periphyton may not be used as a food source. This was also indicated by the great variability in *H. sparna* abundances amongst sites and streams which did not strongly correlate with the potential nutrient sources measured. This is in contrast to Fairchild & Holomuzki (2002) who found *H. sparna* positively associated with total seston (which included algal and animal material), and it was predominant downstream where detrital (leaf) loads were high. This species was found to have low seston utilization, as 80% of all food eaten was egested (Ross & Wallace 1983).

4.4.3.2 *Hydropsyche slossonae*

Abundances of *H. slossonae* clearly did not follow a decay function and so could not be compared to the derived model. Instead a linear regression was used to compare its changes in abundance with distance downstream. Overall there were significantly higher abundances of *H. slossonae* in downstream reaches, which held true in barren landscapes. Forested landscapes had lower abundances with no significant change in abundance with distance downstream. This only partly agrees with the broad survey in Chapter 2, where higher abundances in barren landscapes were also found, with no difference from outlet to downstream, whereas forested landscapes had higher abundances downstream unlike results in this chapter.

Lower downstream abundances in sampling time three and four were partly attributable to the presence of young, unidentifiable larvae. Before these early instars were present at downstream reaches, sampling time one and two, there were significant increases in abundances of *H. slossonae* downstream. If adults oviposited upstream, once early instars started to develop into identifiable larvae they could have been enumerated as *H. slossonae* and then there would have been no changes in abundance with distance from the outlet because of increased abundances upstream. Subsequent drift may have evened out larval abundances over time.

Distributions within and among streams were highly variable. Combining all barren streams, there was a significant decline in abundances of *H. slossonae* with increasing distance downstream. When streams were analyzed individually, this trend only held true for one barren stream, Watern, whereas in Above Hatchet downstream abundances increased and in Portugal Cove there was no significant change. In the two

forested streams where *H. slossonae* was present in sufficient numbers to analyze, abundances declined downstream but this difference was not significant. Overall, *H. slossonae* had slightly higher abundances near outlets that declined downstream, but in general this species occurred throughout Newfoundland streams.

Hydropsyche slossonae had a broad longitudinal distribution and appeared to be able to exploit a range of resources and have a wide tolerance for environmental conditions. The distribution of *H. slossonae* elsewhere is also broad. In a spring-fed Minnesota stream, it occurred throughout the two km section sampled with higher abundances below outlets (MacKay & Waters 1986). Its distribution was similar in warmer Ontario streams with greater abundances below outlets where temperatures were higher than similar habitats in Minnesota streams, indicating temperature may influence its growth rate (MacKay 1986). A similar distribution was observed in a northern Michigan stream, where *H. slossonae* occurred throughout a five km section with abundances greatest below an outlet. It was able to utilize a range of substrate sizes with differing *Cladophora* cover (Fairchild & Holomuzki 2002) and this generality may also contribute to its occurrence throughout Newfoundland streams.

This species had no strong correlation with any of the physiochemical variables measured, nor with phytoplankton or zooplankton abundance. This was unlike results of Fairchild & Holumuzki (2002) who found a positive association with algal seston quantity. The lack of correlation to any of the factors measured here permits further speculation as to reasons for its distribution here. However, the longitudinal distribution of this species here was similar to that of periphyton abundance indicating this may be a

utilized food source or substrate type in Newfoundland streams as Kerans (1996) found fewer *H. slossonae* drifted from periphyton-covered substrates than periphyton-absent substrates. Overall, *H. slossonae* appears to be a generalist, able to inhabit riffles throughout a stream.

4.4.4 Physiochemistry, plankton and periphyton

There were significant relationships amongst pH, conductivity, temperature, velocity, abundances of periphyton, phytoplankton and zooplankton and abundances of individual species. However, regression coefficients were weak as discussed in the above sections on individual taxa. An experimental approach is needed to advance our understanding of the relation between hydropsychid occurrence/abundance with these environmental factors.

Transplant experiments using flow-through channels within a stream and among streams, as outlined by Bourassa & Cattaneo (2000), would be one approach to the assessment of larval growth and survival with different seston compositions. Another method is the tracking of trophic relationships in streams using radiolabeled particles (Rounick et al. 1982). Monoghan et al. (2001) radiolabeled natural detritus and diatoms to determine their longitudinal removal rate by *Hydropsyche* and blackfly larvae and found there was no significant uptake of the material. Dyes have also been used to measure nutrient uptake per unit time or to measure nutrient spiraling (Wotton et al. 1995; Wotton et al. 1996). Recently, stable isotope analysis has been used to track trophic relationships, relying on differences in the uptake of lower (^{12}C & ^{14}N) and higher (^{13}C & ^{15}N) mass isotopes and the C:N ratio to track the flow of nutrients (Kendall et al. 2001).

This technique has not been used with hydropsychids. Another tool is lipid analysis where the presence of certain lipids indicates the use of given food sources by an organism. This technique will be explored later in this thesis.

4.4.4.1 Periphyton

There is an array of terminology describing periphyton (Weitzel 1979). In this study periphyton was defined as all organisms containing chlorophyll-a which grew on and were attached to the tile samplers. Extracting chlorophyll-a is a practiced method (Cushing et al. 1983) for estimating periphyton quantities as outlined by Eaton et al. (1995b). In this study, periphyton abundances were used solely as a method of comparison among streams. This method was not meant to estimate primary production as this can be highly variable (Clark et al. 1979). Chlorophyll-a measurements are affected by the age and physiology of cells, light intensity, photoperiod, shade adaptation capabilities, community composition and nutrient deficiencies (Clark et al. 1979). Also many factors affect periphyton growth including light availability, transparency of the water column, pH, nutrients (nitrogen, phosphorous, carbon), dissolved materials (calcium, sulphur, silicon), trace metals (e.g. iron, copper, selenium) and temperature (Clark et al. 1979; Lowe 1979; Weitzel 1979).

Dudley et al. (1986) gave three functions of periphyton in streams, 1) as a food source for herbivores, 2) changing the physical nature of the substrate and 3) competing with invertebrates for substrate space on which to attach. Hydropsychids are generally filter feeders however they also graze on periphyton (Erichsen-Jones 1950; Winterbourn & Harding 1993), particularly over the winter months when it has been suggested that

nets are not constructed (Fuller & MacKay 1980a; Rhame & Stewart 1976; Winterbourn & Harding 1993). The presence of periphyton influences the density of hydropsychids, both positively by providing stable substrates, a refuge from predators (Barber & Kevern 1973; Dudley et al. 1986; Holomuzki et al. 1999; Williams & Hynes 1973) and decreasing drift periodicity (Kerans 1996) and negatively because of decreased currents (Gregg & Rose 1985) and increased periphyton biomass (Bourassa & Cattaneo 2000). Towns (1981) found that hydropsychids used filamentous moss as a stable substrate, but that filaments interfered with filtering abilities of their nets. Englund (1993) found fourth instar larvae able to utilize the spacing between filaments of a moss (*Fontanalis sp*), also found in Newfoundland streams, but filaments were too closely spaced for utilization by fifth instar larvae. Thus it is not surprising that the general measure of periphyton abundance used here was not closely related to abundances of some species of hydropsychids in this study.

The chlorophyll-a estimate of periphyton remained relatively constant from outlets to downstream as did abundances of *H. sparna* and *H. slossonae* in most streams but only weak direct correlations were found. This was also the case in other studies. Harding (1997) found that periphyton abundance peaked in mid reaches of a New Zealand stream, which correlated with the abundance of one hydropsychid species present. In an experimental field study, Bourassa & Cattaneo (2000) manipulated total phosphorous and light levels in flow-through channels and monitored them for 55 days. They found that in shaded streams single cell and colonial forms of attached algae dominated and the predominant filter feeders were Simuliidae, whereas in open streams

filamentous and chain-forming attached algae were prevalent and there was a shift in the population towards grazers. However, overall hydropsychids were not significantly affected by the manipulations. Thus the nature of this resource does not appear to significantly influence hydropsychid distribution. However, because hydropsychids graze this material (Erichsen-Jones 1950) there may be an affinity for only certain species, thus there would not be a clear relationship with periphyton abundance in general. The species composition of Newfoundland periphyton is diverse (Thompson 1987), so there is potential for a variable hydropsychid response (Sheath & Cole 1992).

Quantities of periphyton were generally higher at forested outlets compared to barren outlets. Available nitrogen and phosphorous content of soils are generally higher in forested than in barren landscapes (Heringa 1981) and leaching of these nutrients into streams could account for differences in periphyton abundance. Barren streams are also more acidic which may affect periphyton abundances (Jamieson 1974; Weitzel 1979). In forested landscapes the amount of periphyton decreased downstream, and in the smaller streams this could be because of shading of the stream by overhanging vegetation. In barren streams with no shading, periphyton quantities slightly increased downstream.

There was no difference in the chlorophyll-a content amongst sampling times. Periphyton abundances may have increased in the summer months because shallower water, declining velocities and less turbidity allowing more light penetration would have promoted periphyton growth and accumulation (Pryfogle & Lowe 1979). In the streams studied here, turbidity was likely low because of minimal human land use and vegetated drainage basins curtailing sedimentation.

Communities on artificial substrates are generally similar to natural communities (Weitzel et al. 1979). Two weeks is the optimal time for tile exposure with exposure greater than 4 weeks leading to greater debris accumulation and increased biomass of faster growing species (Weitzel et al. 1979). In this study, tiles remained in streams for a set time period (three weeks) to control the amount of time for colonization and biomass accumulation, assuming analogous rates amongst streams. Thus the data collected probably was a good index of the natural periphyton abundance.

4.4.4.2 Phytoplankton

Abundances of phytoplankton showed a significant decline with increasing distance downstream, but the rate of decline was very slight (-0.0827) indicating that abundances decreased only slightly from outlets. Abundances were robust with landscape but differed amongst streams. Seven of the eight streams showed a decline, but Portugal Cove, a large barren stream, had a slight increase possibly because of material coming in from fens beside the stream.

Other studies have measured the chlorophyll-a content of the phytoplankton finding no significant difference (Harding 1997) or a reduction downstream (Hoffsten 1999; Maciolek & Tunzi 1968). The latter authors showed declining levels occurred over a distance of 1.9 km or more, while other than Portugal Cove in this study, all samples were collected over a shorter distance and showed a slight decrease. Periphyton might also contribute to the seston by being sloughed off the bottom and so chlorophyll-a would have been a poor tracer of lake phytoplankton influence along the stream (Vadeboncoeur 1994).

In studies on single stream systems, no correlation has been reported between chlorophyll-a content of the phytoplankton and hydropsychid abundance as phytoplankton declined faster than hydropsychid abundance (Bronmark & Malmqvist 1984; Oswood 1979). In those studies phytoplankton must not have been a limiting resource for hydropsychids. This study contradicts the general trend of phytoplankton rapidly declining as the overall rate of decrease (-0.0772) was not rapid and was much lower than that of hydropsychids (-0.2008). This result indicated that hydropsychids were not causing a steep decline in phytoplankton abundances or their rates of decline would have been similar. Monaghan et al. (2001) calculated the longitudinal loss rate of fine particulate organic matter (FPOM) from the seston and found *Hydropsyche* removed $0.21\%m^{-1}$ accounting for ~3% of the total stream deposition. Therefore hydropsychids were not regulating the downstream availability of seston. Although the reported 3% was a low proportion of the total (Monaghan et al. 2001), high abundances of hydropsychids at outlets do contribute to increased nutrient retention through spiraling (Elwood et al. 1983). Low assimilation efficiencies of FPOM result in much of this material becoming fecal pellets which are colonized by micro-organisms (Wotton et al. 1996; Wotton et al. 1998). Pellets are larger and denser than FPOM and settle out of the water column where they are more readily captured by other organisms. This process reduces the downstream transport of nutrients (Malmqvist 2002). In this study, it was possible that material produced at outlets was reutilized by downstream populations, which may have accounted for increased abundances of *H. sparna* at station three as it is a generalist feeder (Richardson & MacKay 1991) and could capitalize on such a resource. Watern did

show a significant relationship between *C. pettiti* at the outlet and *H. sparna* at station three (Figure 4.42), suggesting the need to evaluate this possibility.

Hydropsychid abundances generally showed a similar rate of decline to phytoplankton abundances in Beaver Pond, Split Rock and Above Hatchet, meaning that the rate of phytoplankton decline was higher compared to the other streams. Hydropsychids may have an affinity for certain groups of phytoplankton, resulting in increased removal of these species which may have been a larger proportion of the seston in these streams. Another possibility is the lack of other nutritional resources in these systems, causing a heavier reliance on phytoplankton. To sufficiently observe changes in phytoplankton abundance all filtering taxa would need to be assessed. For example, there are large populations of simulids, philopotamids and polycentropids in Newfoundland streams (Larson & Colbo 1983; Lomond & Colbo 2000; McCreadie et al. 1995) which also utilize this resource. To fully investigate trends in phytoplankton abundances, nutrient spiraling and the use of this resource by filter feeding guilds needs to be considered.

4.4.4.3 Zooplankton

Zooplankton abundances showed very rapid declines below outlets in four of the eight streams abundant zooplankton flowed from lakes, with much higher rates than predicted by the model with the exception of Broad Cove. The other four streams had low abundances at outlets, and abundances remained low throughout the longitudinal sections sampled. Zooplankton abundances were reported to decrease with increasing distance from outlets (Chandler 1937; Voshell & Parker 1985). Higher abundances of

hydropsychids at outlets have been attributed to higher amounts of animal material in the seston (Cushing 1963; Petersen 1987c). Fourth and fifth instar hydropsychids remove higher proportions of animal material from the seston compared to the natural proportion of this material in the water as shown by gut content analysis (Benke & Wallace 1980; McCullough et al. 1979; Ross & Wallace 1983). The removal of zooplankton by high densities of hydropsychids can alter seston quality (Ross & Wallace 1983). Zooplankton has a high nutritional value because of higher assimilation efficiency from its protein content (~70%) in comparison to plant food sources such as phytoplankton (diatoms ~30%) and detritus (~10%) (Benke & Wallace 1980; Fuller et al. 1988; Parker & Voshell 1983; Valett & Stanford 1987). In a laboratory feeding trial, Petersen (1987a) fed *Hydropsyche* detritus, macroalgae and zooplankton. Larvae stopped ingesting detritus, then macroalgae but continued consuming zooplankton, showing selection of this food source. Thus high densities of hydropsychids at outlets are sustained by high inputs of zooplankton and that zooplankton is quickly removed from the seston limiting its downstream transport (Chandler 1937; Cushing 1963; Ross & Wallace 1983; Voshell & Parker 1985).

In this study zooplankton concentrations were elevated at some outlets but quickly declined. Comparisons to the literature were difficult as rates of decline were often not quantified and distances sampled downstream were much greater than those in this study (Cushing 1963; Hoffsten 1999; Oswood 1979; Valett & Stanford 1987).

Cheumatopsyche pettiti abundances generally declined at a faster rate than that of zooplankton. This species has the smallest capture net mesh size of Newfoundland

hydropsychids and may feed on smaller particles. The slopes of the decline in abundance of *H. betteni* and zooplankton were not significantly different and so this species may consume zooplankton as it is reported to feed on animal material (Coffman et al. 1971; Fuller et al. 1988). However, *H. slossonae* is also reported to be carnivorous (Coffman et al. 1971; Shapas & Hilsenhoff 1976) but as its abundance showed little association with outlets they obviously do not depend on lentic zooplankton. The abundance of all hydropsychids and the zooplankton abundance had similar rates of decline, indicating that at the family level these filter feeders likely feed on zooplankton. The clear relationship between zooplankton and *H. betteni* and total hydropsychid abundance has not been shown statistically before. Most studies imply correlations but have not used an ANCOVA to demonstrate the similarities in slopes of abundances along streams. The statistical relationship however does not necessarily mean a feeding relationship. For example, changes in seston abundance are linked to discharge (Vadeboncoeur 1994) which also relates to hydropsychid abundance (Sharpe & Downes 2006).

Zooplankton was sampled only once at each site during each sampling period, giving only a brief glimpse at the zooplankton population of the stream. Zooplankton abundances are temporally dynamic (Eriksson 2001), so more extensive sampling would provide a better quantitative estimate of the long term availability of seston for the larvae. This data may have altered the rate of decline of the zooplankton and hence the strength of its relationship with hydropsychid abundance.

Downstream declines of zooplankton abundances were not only attributable to consumption as individuals may settle out or be caught in turbulent eddies (Eriksson

2001). This not only alters abundances but also zooplankton community composition as Eriksson (2001) found the average body length of zooplankton decreased downstream as smaller zooplankton withstood settling out of the water and were carried further downstream. Outlet morphology also affected zooplankton composition with *Bosmina* found further downstream when outlet littoral zones were deeper (>2 m deep) and cyclopoid copepods when they were shallower (<1 m deep) (Walks & Cyr 2004). In four streams lentic zooplankton was eliminated within 1 km of outlets and the greatest reductions occurred within the first 50m due to high densities of filter feeders, including hydropsychids (Walks & Cyr 2004).

In this study, zooplankton community composition was not considered but it could account for weak correlations with hydropsychid abundance. For example, *Cyclops* was able to escape *Hydropsyche* nets 60-80% of the time because of its strong swimming abilities and larger zooplankton had longer handling times which allowed smaller zooplankton to escape *Hydropsyche* nets (Eriksson 2002). Hydropsychids are tactile predators and so large prey may be easier to detect, particularly if they bounce against hydropsychid nets as this elicits a quick response (Eriksson 2002). In addition, hydropsychids rely on currents to sweep prey into their nets, and zooplankton tend to drift in the upper water column and so may not be available for uptake (Hoffsten 1999). Zooplankton is also more abundant in the spring during phytoplankton blooms and thus both are more available as food when larvae are developing into fifth instars which require high energy stores for pupation, reproduction and adult activity (Benke & Wallace 1980).

Ross & Wallace (1983) found hydropsychids selectively removed animal material from the seston, as the proportion in their gut was much higher than that naturally occurring in the seston. Other studies showed gut contents of hydropsychids to contain chironomid, blackfly and mayfly larvae (Basaguren et al. 2002; Erichsen-Jones 1950) which were generally not part of the seston collected here, but these organisms drift often at night (Wiley & Kohler 1984). Drift occurs when benthic organisms move into the water column and are carried downstream, a mechanism used to avoid predators and competition (Hoffsten 1999). Drift samples were not collected at night in this study but this would be an important addition to future Newfoundland studies coupled with gut analysis which may result in better modeling of animal food availability and hydropsychid abundance.

4.4.5 Temperature probes

Temperature probe data clearly showed that outlets were warmer than downstream sites. Only Broad Cove had temperatures previously recorded (1979-1980) with similar findings to those of this study (Larson & Colbo 1983). *Arctopsyche ladogensis* rarely occurred at outlets and is recorded to occur in cooler waters elsewhere (Englund et al. 1997). The influence of temperature on the distribution of *H. alternans* and *D. modesta* is not known (Cudney & Wallace 1980). Table 1.3 shows that *C. pettiti* and *H. betteni* occur in warmer waters elsewhere, which may influence their Newfoundland distribution as these species were found in greater abundances near the warmer outlets. *Hydropsyche slossonae* occurs in cooler waters but is tolerant of warmer temperatures (Table 1.3) and was found throughout stream reaches here. *Hydropsyche*

sparna has a broad temperature tolerance (Table 1.3) so occurs throughout a stream from warmer outlets to cooler downstream reaches as found in this study.

Forested streams were warmer overall than barren ones. This may be because of small-scale climate differences created by altitude, nature of the surrounding terrain and proximity of the ocean (Ecological Stratification Working Group 1996)(section 1.10). Forested streams had decreased exposure to cool winds and fog which would decrease water temperatures compared to barren streams (Larson & Colbo 1983)(see section 1.10). These conditions were prevalent on the southern Avalon Peninsula where Above Hatchet, Watern and Portugal Cove were located.

Parapsyche apicalis is known to occur in colder streams (Flint 1961), which was also demonstrated in Newfoundland. (Wiggins 1996)The streams with *P. apicalis* were presumably ground water fed as their water temperatures were cooler throughout the summer. This species was found in lab trials to have reduced survival at higher temperatures (Chapter 3) and water temperature was a factor influencing its distribution in Newfoundland. Oxen Pond initially appeared as an anomaly as *P. apicalis* occurred near the outlet, a site where it generally was not found (Chapter 2). Temperature probe data showed water temperatures were elevated at the outlet but declined downstream, presumably because of ground water input as springs are common around Oxen Pond (Larson & Colbo 1983).

Small increases in mean temperature result in higher production and development rates of stream organisms (Cudney & Wallace 1980; Hildrew & Edington 1979), which is true for hydropsychids below outlets (MacKay & Waters 1986). *Hydropsyche slossonae*

exhibited a similar life cycle in warm and cool streams because of its broad temperature tolerance (MacKay 1986). However, *C. pettiti* had a higher tolerance for warmer temperatures than *H. slossonae* (MacKay 1986). This difference in tolerance with *C. pettiti* may influence abundances of *H. slossonae* at Newfoundland outlets.

4.4.6 Effectiveness of Modeling

Utilization of a decay model for establishing sampling stations proved useful for comparisons across streams. Prior studies on longitudinal changes did not have a strong rationale for site selection beyond choosing more sites closer to an outlet (Eriksson 2001; Oswood 1979). Some studies sampled two or three sites close to an outlet and then a single site over a kilometre downstream and so the gradual change in the hydropsychid community was not revealed (Oswood 1979; Valett & Stanford 1987). Most studies were conducted in a single stream system (Bronmark & Malmqvist 1984; Cushing 1963; Oswood 1979; Valett & Stanford 1987), with the exception of Eriksson (2001) where site selection was based on riffle availability in four streams. In this study it was difficult to accurately measure distances from outlets, but in future the use of a Global Positioning System (GPS) could greatly improve accuracy. Distances given by the model did not always fall directly in a riffle habitat and so the nearest riffle was chosen, but this was always nearby as streams generally had steep relief profiles..

The rate of decline of -0.5878 accurately modeled the change in abundance of outlet species of hydropsychids. *Cheumatopsyche pettiti* abundances generally had a steeper rate of decline than -0.5878, but it did fit the model in four of the eight streams. The rate of decline of *H. betteni* was similar to this iteratively derived rate in three of the

four sampling times. Thus the use of the model gave a reasonable approximation for species that had high abundances at Newfoundland outlets.

One strength of the model is its use as a comparative tool to investigate changes in the hydropsychid community across scales of landscape, time (sampling time) and space (multiple streams). This would be useful for monitoring changes in life history patterns, abundances and distributions because of environmental changes over time and space. Throughout this study, statistically comparing the derived model with predicted models provided a novel basis for a null hypothesis. The null model was no difference in the slopes between the derived model and the predicted model. If accepted, this indicated that the derived model adequately modeled the population. The null model was in fact rejected most of the time, but was a novel investigative tool into the longitudinal change in hydropsychid communities. Following that, a more accurate slope could now be developed for Newfoundland streams as a method for sampling hydropsychid communities.

The use of a negative power function was originally developed for prediction of blackfly abundance below an outlet (Sheldon & Oswood 1977) and was tested against abundances of hydropsychids (Oswood 1979) and seston concentration (Vadeboncoeur 1994). Oswood (1979) concluded that hydropsychid abundances generally agreed with the model. His data were log transformed and fitted to a linear regression using an ANOVA with a normal error structure which was found to fit the data. However, in the present study a more robust error structure was needed because of the high variability in hydropsychid counts. This variability may be because this study was replicated in space

and time, while the original test of the negative power function was conducted in a single stream that was only sampled once. The original model was based on the assumption that there should be a proportional relationship between filter feeders and their food supply and yet this was never tested statistically, only compared by observing general trends. A strength of the model in this study was its ability to statistically test the longitudinal distribution of entities (i.e. total hydropsychids and zooplankton).

Hydropsychid larvae were not the only filter feeders at outlets in this study. Other filter feeders commonly present in Newfoundland streams were blackfly larvae (McCreadie et al. 1995), the chironomid tribe Tanytarsini (e.g. *Rheotanytarsus*), and other caddisfly taxa such as *Dolophilodes, Chimarra, Wormaldia* and *Polycentropus* (Genge 1985; Lomond & Colbo 2000). These organisms also removed seston and thereby influenced transport of this material downstream and may have partially accounted for the lack of correlation between the abundance of the seston and hydropsychids in this study. High densities of many types of filter feeders were found to remove a significant portion of the seston in four Ontario streams (Walks & Cyr 2004) supporting the importance of the composition of the filter feeding guild. Models should be tested using all filter feeders and the relative contribution of the instars within these groups as the amount of material removed is proportional to filter feeder abundance, capture efficiencies of their nets and size fractions filtered (Wallace & Merritt 1980). Thus other filter feeders were competing with hydropsychids for food resources. However, rarely does a lack of food impinge on the growth of hydropsychids as McCullough & Minshall

(1979) found filter feeders (blackflies and *Hydropsyche*) to remove ~1% of the available seston per day.

4.5 Conclusion

Deriving a model provided a basis for comparison of the hydropsychid community, plankton and periphyton amongst streams of different sizes. The process allowed for a better comparison among streams and landscapes and permitted exploration of temporal shifts in hydropsychid abundances. A more accurate model could consequently be derived to investigate effects of outlets on the hydropsychid community and on seston transport in Newfoundland streams. The most rapid changes in the community occur near outlets. Abundances of *C. pettiti* and *H. betteni* declined rapidly below outlets, *H. slossonae* had a fairly constant longitudinal abundance and *H. sparna* increased downstream. Longitudinal periphyton abundances were relatively constant, and phytoplankton and zooplankton abundances declined downstream. This study showed similar longitudinal trends in zooplankton and hydropsychid abundance, based on statistical comparisons which were essential to improving understanding of these interactions. It is not known if hydropsychids were responding to the quantity of food as strong linear correlations were not evident. Higher temperatures were seen at outlets and in forested stream systems which may account for higher hydropsychid abundances in these places. *Parapsyche apicalis* was restricted to cooler streams in Newfoundland. Overall quantities of food did not explain the change in the species' composition of the community. Although, correlations with zooplankton indicate food quality may be an

important influence on hydropsychid communities. This hypothesis is supported in the literature (Bronmark & Malmqvist 1984; Ross & Wallace 1983; Valett & Stanford 1987) with subtle differences in resource utilization possibly causing changes in species' abundance (Alstad 1987). Stronger correlations may exist when these relationships are investigated at a finer scale.

5. CHAPTER 5: LIPID AND FATTY ACID COMPOSITION OF HYDROPSYCHIDAE LARVAE (FILTER-FEEDING TRICHOPTERA)

5.1 Introduction

Hydropsychid species distribution and abundance differs among streams and it is not known if this is influenced by food resource use. Newfoundland hydropsychids occur across a range of stream habitats with individual species having more restricted distributions within and among streams. In Newfoundland streams, larval abundances are generally greater at lake outlets with a marked decline downstream. This trend is similar in forested and barren landscapes, although forested streams generally have higher hydropsychid abundances (Chapter 2 & 4). It was shown that the potential food resources, phytoplankton, zooplankton and periphyton, changed from outlet to downstream along with hydropsychid occurrence/abundance (Chapter 3 & 4), but to what extent does the latter depend on the former? The existence of multiple species of hydropsychids in a given stream reach has partly been attributed to partitioning of food resources via net mesh-size differences between species and instars (Cummins 1973; Edington et al. 1983; Wallace 1975b; Wallace 1975a). However, the high degree of omnivory amongst species (Alstad 1987), and the many other factors influencing their distribution (Chapter 1) has led to a debate about how far food resources influence hydropsychid distribution and abundance. The focus of this chapter is an exploration of the feeding ecology of Newfoundland hydropsychids in terms of location (outlet versus downstream) and landscape (forested versus barren) using the tool of lipid analysis to examine the utilization of food resources by hydropsychids to address whether: 1) lipids can be used to discriminate food resources in lotic systems, 2) species are partitioning

food resources, and 3) the influence of food resources on species' distribution and abundance in terms of location and landscape.

Animal material is a large portion of hydropsychid diets (Haefner & Wallace 1981) (see Chapter 1 and Appendix 4 (section 10.4)) but is not a large portion of the seston (McCullough et al. 1979). Therefore questions arising are: do hydropsychids selectively remove animal material from the seston? Are they utilizing a greater proportion of certain species of plankton and so responding to the seston at a finer scale than overall quantity?

Determining if hydropsychids are differentially removing material from the seston requires extensive knowledge of larval food ingestion, digestion and assimilation in comparison to the composition of the seston. Larval food ingestion has traditionally been investigated using gut content analysis which shows the type and approximate volume of food ingested. However, accurate identification of fragmented and partially digested material is difficult and often more than half of gut material is classified as detritus because it is unrecognizable as plant or animal material (Benke & Wallace 1980; Fuller & MacKay 1980a). A further problem with this technique is that it is impossible to know if a sclerotized head capsule, for example, once contained animal material that was ingested or if microbial film coating its surface was used as a food source. Consequently, gut content analysis lacks accuracy, is time consuming, and only considers food recently ingested since hydropsychids have a gut clearance time of about two hours (Sangpradub & Giller 1994).

Gut content analysis showed *A. ladogensis, P. apicalis* and *H. betteni* to be highly carnivorous, while other species which occur in Newfoundland had generally lower but varying proportions of animal material in diets (see reviews in Chapter 1 (section 1.8) and Appendix 4 (section 10.4)). There are generally two sources of animal material in Newfoundland streams, zooplankton from lakes and animals drifting downstream.

Given all the above difficulties associated with gut content analysis, and the time and expertise needed to identify and quantify the diverse array of potential food resources in the water and those ingested by the hydropsychids, it was decided to explore the use of lipid analysis in lotic systems. Lipid analysis has been used for tracking trophic relationships in marine (Budge et al. 2002; Cripps & Atkinson 2000; Smith et al. 1997; Stevens et al. 2004a) and lentic (Kainz et al. 2004; Kiyashko et al. 2004; Sekino et al. 1997; Sushchik et al. 2003) environments and was adapted to this lotic study in order to assess the fatty acid composition of a family of filter feeders.

Little is known of the lipid content of stream macroinvertebrates, except for a few studies comparing families or higher levels of classification (Bell et al. 1994; Hanson et al. 1985; Meier et al. 2000; Sushchik et al. 2003). Nor is there much known about the lipid composition of closely related freshwater species in a feeding guild (Goedkoop et al. 1998; Sekino et al. 1997). Despite the important role hydropsychids serve in the lotic foodweb (see section 1.5), little is known of their fatty acid composition. To provide context for this research a brief background to lipids and fatty acids is given in Appendix 3 (section 10.3), as well as a review of hydropsychid feeding ecology in Appendix 4

(section 10.4) and a summary of fatty acid markers in freshwater ecosystems in Appendix 5 (section 10.5).

5.1.1 Lipids and fatty acids

Fatty acids of aquatic organisms have a varying degree of saturation, are typically between 12 and 24 carbon atoms long and account for ~2-15% of the dry weight of the organism (Napolitano 1999). Fatty acids can be used as fatty acid markers through the food web because some are limited or dominant in certain groups of taxa. If fatty acids retain their basic structure after consumption, they can be used to trace consumptive pathways through food webs and can indicate sources and sinks of organic material (Napolitano 1999). The adipose tissue of an organism contains fatty acids derived from its diet over time (Napolitano 1999), which may allow one to determine the 'fatty acid signature' for a species.

Being able to decipher time-integrated dietary intake is important when considering the food partitioning of multiple species of filter-feeding caddisflies. Gut content analysis previously used for this is unsatisfactory for reasons explained above. Fatty acids may allow a more precise rendition of the diet of Hydropsychidae, because fatty acid markers can potentially identify the source(s) of nutrients.

Fatty acid composition has been used to determine inter- and intraspecific differences among closely related aquatic species, including those belonging to the same family (Auel et al. 2002; Budge et al. 2002; Falk-Petersen et al. 2000; Goedkoop et al. 1998; Jayasankar & Kulandaivelu 1999; Sekino et al. 1997). The majority of these studies considered algae, zooplankton or sediment-feeding chironomids, except for

Budge et al. (2002) where marine fish and invertebrates were investigated. However, the invertebrates were large, commercially important species including lobster, crab and shrimp (Budge et al. 2002). Auel et al. (2002) found two con-generic species of arctic hyperiid amphipods to have different diets. However, one occurred deep in the ocean and the other near the sea ice. Similarly two larval lake chironomids, *Chironomous anthracinus* and *C. plumosus,* sampled from the same depth showed differences in diet. In this case, one was a suspension-feeder with higher proportions of a diatom fatty acid marker and the other was a detritus-feeder with higher proportions of bacterial fatty acid markers (Goedkoop et al. 1998). Therefore it is possible to distinguish the diet of freshwater macroinvertebrates.

If hydropsychids are opportunistic feeders, switching from one food source to another, gut content analysis would not reflect their diet over time. Fatty acid markers would better integrate the various sources of food consumed over time. This in turn would allow comparison both between species and within a species in different areas, different landscapes, different streams or different locations within the same stream. Questions addressed in this section were: 1) Do different species have significant differences in their fatty acid composition? 2) Can this be related to the types of foods consumed? 3) Do different species taken from the same location in a stream show food resource partitioning? 4) Does the diet of a given species differ with landscape, stream or locations in a stream?

5.2 Materials and Methods

Larvae and pupae were hand picked from substrates in ten streams on the Avalon Peninsula of Newfoundland (Figure 5.1). At each site, 25 or more individual larvae and pupae were collected if possible in order to obtain at least two samples of five fifth instar individuals per species where multiple species were known to co-exist. Sampling occurred in mid May to early June 2004 and was repeated in mid to late August. *Parapsyche apicalis* was collected in late June 2005. They were transported live, on ice, to the laboratory where they were identified to species with the aid of keys (Rutherford 1985; Schefter & Wiggins 1986; Schuster & Etinier 1978).

Whole specimens of pupae were used. For larval samples, to avoid contamination with gut contents, the head and anal end of each larva were cut off and the gut was pulled out (Glasgow 1936). Then they were placed in lipid cleaned test tubes. For larvae, five individual fifth instars were pooled per sample, with six or more individuals of fourth instars pooled per sample. Individual pupae or pooled samples of two to four were used depending on the number collected of a species. Approximately 2 mL of chloroform was added and the tubes were capped under nitrogen, sealed with Teflon tape and kept frozen until extraction. A summary of samples collected is given in Table 5.2. A sample hereafter refers to pooled individuals as per the above methods.

5.2.1 Study area

Eight streams were selected on the Avalon Peninsula (Figure 5.1); four from forested landscapes and four barren landscapes as previously described (Table 2.1, Chapter 4) (Appendix 2 (section 10.2)). Streams were also chosen based on width at the

lake outlet, giving a gradient from small to large streams (Table 5.1). Streams were sampled at the outlet (equivalent to outlets/Station 0 in Chapter 4) and downstream (equivalent to Station 8 in Chapter 4), hereafter referred to as location. Two additional streams, Goat Cove and Nagles Brook, were sampled because one species, Parapsyche apicalis, was not found initially. These streams did not have outlets and widths were measured at the collection sites (Table 5.1).

Table 5.1 Streams sampled, with landscape type and size descriptions.

Stream Name	Abbreviation	Landscape	Outlet Width (m)
Barking Kettle	BK	Forested	1
Great Pond	GP	Forested	1.5
Broad Cove	BC	Forested	3.5
Beaver Pond	BP	Forested	14
Split Rock	SR	Barren	1.5
Above Hatchet	AH	Barren	1.75
Watern	WT	Barren	2.5
Portugal Cove	PC	Barren	23
Nagles Brook	NB	Forested	2.2
Goat Cove	GC	Forested	5.5

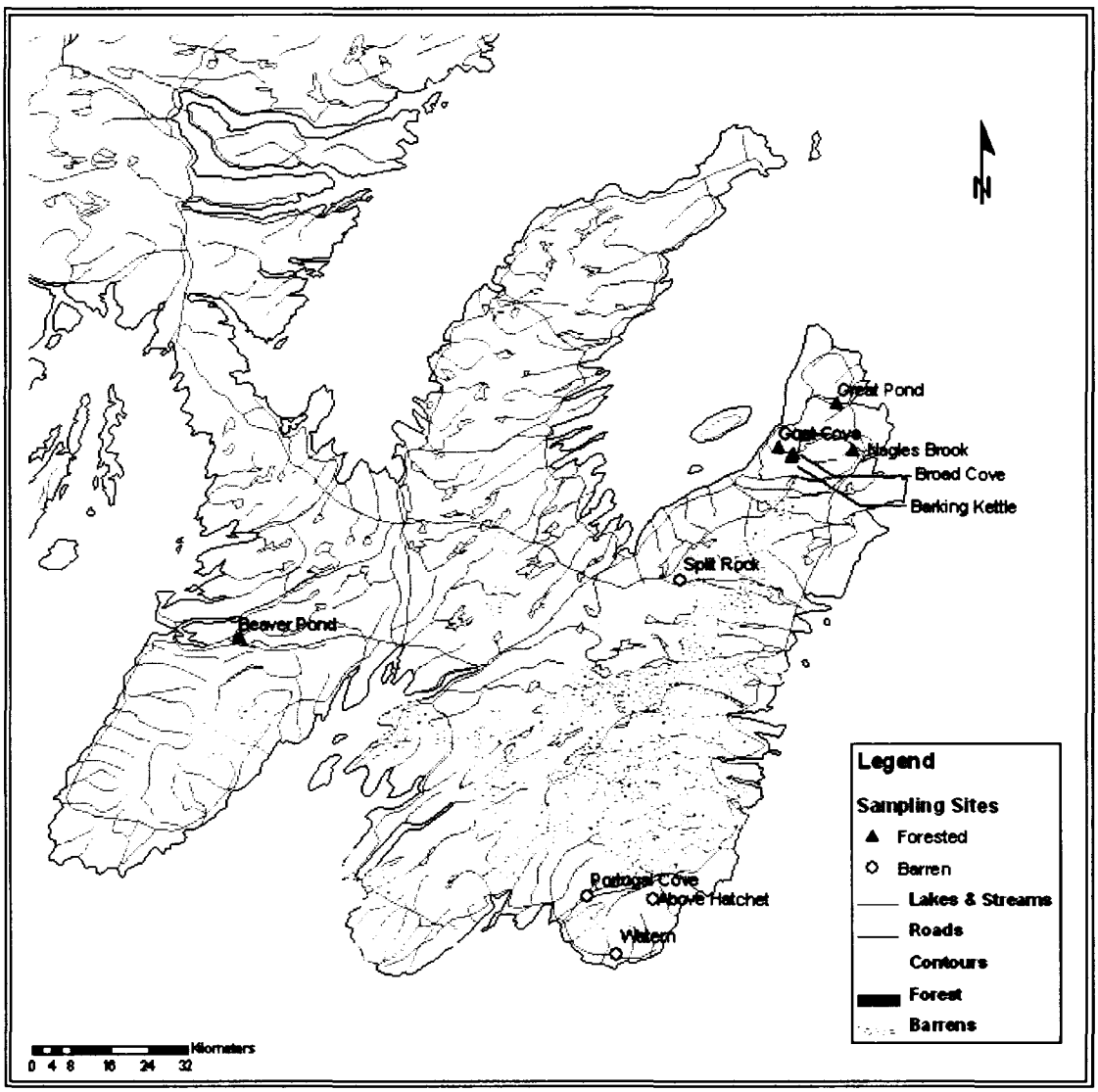

Figure 5.1 Map of the locations of the streams sampled on the Avalon Peninsula of Newfoundland, Canada.

Table 5.2 Summary of samples (pooled) collected by stream and season. Samples were collected in spring and summer 2004 (but *P. apicalis* in 2005). Summer samples in square brackets. A total of 214 samples were collected. lo=outlet, ds=downstream, nb=Nagles Brook, gc=Goat Cove

		Spring Sampling [Summer Sampling]																
		Barking Kettle		Great Pond		Broad Cove		Beaver Pond		Split Rock		Above Hatchet		Watern		Portugal Cove		
Species	Life Stage	lo	ds	lo	ds	lo	ds	lo	ds	lo	ds	lo	ds	lo	ds	lo	ds	Total
A. ladogensis	larvae								[2]						3 [1]		[1]	7
	pupae														3		3	6
C. pettiti	larvae	3 [2]		1 [1]		1 [2]						3 [2]		5 [7]		1 [5]		33
	pupae	3										2		[3]		[3]		11
D. modesta	larvae		4 [1]	[1]	1													7
	pupae		1															1
H. alternans	larvae							6 [4]								3 [3]		16
	pupae															2		2
H. betteni	larvae	6 [2]	2 [1]	6 [6]		3 [4]				3 [4]	1	1						39
	pupae	3																3
H. slossonae	larvae			5	1 [1]				2			1	3 [5]	6 [1]	2 [3]	1 [2]	3 [1]	37
	pupae													1 [1]	[1]			3
H. sparna	larvae		1	[1]	[2]		[2]	1	2	[1]	1 [2]		3	[1]	5 [3]	3	1 [2]	31
	pupae																	0
		NB	GC															
P. apicalis	larvae	7	6															13
	pupae	1	4															5

5.2.2 Lipid analysis

Lipids were extracted in chloroform:methanol (2:1) using a modified Folch procedure (Folch et al. 1957). Samples were ground with a metal rod, washed three times with 3 mL of chloroform and all washes were combined. Samples were concentrated to 1.5 mL, capped under nitrogen and kept at -20°C until further analysis. Details of the extraction procedure are in Parrish (1999).

After extraction, lipid classes were determined using a thin layer chromatography flame ionization detector (TLC-FID). Samples were manually spotted on the silica-coated Chromarods, and then developed and scanned according to Parrish (1999) using an Iatrocan MK V with air and hydrogen flow rates of 2 L/min and 190mL/min respectively. To determine peak identities, a commercial standard was used (Sigma-Aldrich Canada). The resulting three chromatograms were combined and the peaks were cut manually using T Data Scan 3.10. These data were then entered into a spreadsheet (Excel) and the percent lipid composition determined using calibration curves based on commercial standards (Sigma-Aldrich Canada). Lipid classes were only analyzed for a subset of the samples (including larvae and pupae of all species) because lipid class composition was proved similar in all samples.

An aliquot of each sample was taken to form fatty acid methyl esters (FAME) for gas chromatography (GC) analysis. Each aliquot was evaporated to near dryness under nitrogen gas and 0.5mL of hexane and 1.5mL of boron trifluoride-methanol reagent were added. Samples were vortexed and then sonicated for 4 minutes. They were capped under nitrogen and heated to and maintained at 85°C for 1.5 hours. After cooling, 0.5mL of

chloroform extracted water was added, the sample was shaken and 2mL of hexane added. The upper layer containing the FAME was removed and concentrated under nitrogen gas to a volume of 1.5mL, then capped under nitrogen and kept at -20C until GC analysis.

A Varian 3400 GC was used to analyze FAME following Budge & Parrish (1998). The resulting peaks in the chromatograms were identified by comparison with the retention times of 4 standards: 37-component FAME mixture, Bacterial acid methyl esters mix (BAME), PUFA1 and PUFA3 (Supelco) using the Varian Star Chromatography Workstation Interactive Graphics program, version 5.5. Amounts of individual fatty acids are expressed as a mass percentage of total identified fatty acids.

5.2.3 Data analysis

Data were entered into Microsoft Excel. Statistical analyses were carried out with Minitab version 14.1 and with SAS version 9.1. To differentiate fatty acid composition among species several types of analyses were used. PCA was used to visualize differences among species, followed by One-way ANOVAs to determine which fatty acids were significantly different among species (; forward stepwise regression was used to determine which fatty acids were causing the greatest separation among the species; and discriminant function analysis was used to quantify the distance among species. Differences within a species fatty acid composition with respect to season, location, landscape and stream were visualized using PCA, as were differences among species withing a site.

5.3 Results

5.3.1 Lipid Classes in Hydropsychidae

Among the lipid classes, triacylglycerol (TAG) dominated in all species of Hydropsychidae (Figure 5.2). There were no significant differences between lipid classes by species, by life stage, by stream or by landscape. A one way ANOVA of TAG by life stage is shown in Table 5.3. Phospholipids (PL) were found to be significantly higher in the spring samples ($p<0.0001$, Table 5.3), concurrent with significantly decreased levels of free fatty acids ($p=0.005$) and methyl ketones ($p=0.016$). TAG did not differ by season. Outlets had a higher level of TAG ($p=0.001$, Table 5.3) and a lower level of PL ($p=0.015$, Table 5.3) than downstream sites. Because of an interaction with season, location comparisons were conducted for each season separately. Only the spring cohort had a significantly higher level of phospholipids downstream, with the data requiring log transformation. The increase in the proportion of TAG at outlets is balanced with a decrease in the proportion of PL in the spring samples.

Table 5.3 One way ANOVAs for triacylglycerol and phospholipids

One-way ANOVA: Triacylglycerols versus Life Stage

```
Source  DF     SS   MS     F      P
Stage2   1    130  130  0.32  0.573
Error   65  26219  403
Total   66  26348
S = 20.08   R-Sq = 0.49%   R-Sq(adj) = 0.00%
                     Individual 95% CIs For Mean Based on Pooled StDev of 20.08
Level    N   Mean  StDev  ----+---------+---------+---------+-----
larvae  43  64.25  20.08  (-----------*-----------)
pupae   24  67.15  20.09    (---------------*----------------)
                          ----+---------+---------+---------+-----
                           60.0      65.0      70.0      75.0
```

One-way ANOVA: Phospholipids versus Season

```
Source  DF    SS    MS      F      P
Season   1  2601  2601  23.48  0.000
Error   65  7201   111
Total   66  9802
S = 10.53   R-Sq = 26.54%   R-Sq(adj) = 25.41%
                    Individual 95% CIs For Mean Based on Pooled StDev of 10.53
Level    N   Mean  StDev  -------+---------+---------+---------+--
spring  26  23.86  15.55                      (------*------)
summer  41  11.08   5.37  (----*-----)
                          -------+---------+---------+---------+--
                              12.0      18.0      24.0      30.0
```

One-way ANOVA: Triacylglycerols versus Location

```
Source     DF     SS    MS      F      P
Position    1   4106  4106  12.00  0.001
Error      65  22242   342
Total      66  26348
S = 18.50   R-Sq = 15.58%   R-Sq(adj) = 14.29%
                    Individual 95% CIs For Mean Based on Pooled StDev of 18.50
Level   N   Mean  StDev    -+---------+---------+---------+--------
ds     24  54.81  20.81    (---------*--------)
lo     43  71.14  17.10                        (------*------)
                          -+---------+---------+---------+--------
                         48.0      56.0      64.0      72.0
```

One-way ANOVA: log Phospholipids versus Location in Spring

```
Source     DF      SS      MS     F      P
Position    1  0.5309  0.5309  6.99  0.014
Error      24  1.8225  0.0759
Total      25  2.3534
S = 0.2756   R-Sq = 22.56%   R-Sq(adj) = 19.33%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.2756
Level   N    Mean   StDev  ----+---------+---------+---------+-----
ds     10  1.4841  0.2309                (----------*----------)
lo     16  1.1904  0.2992  (--------*--------)
                          ----+---------+---------+---------+-----
                            1.12      1.28      1.44      1.60
```

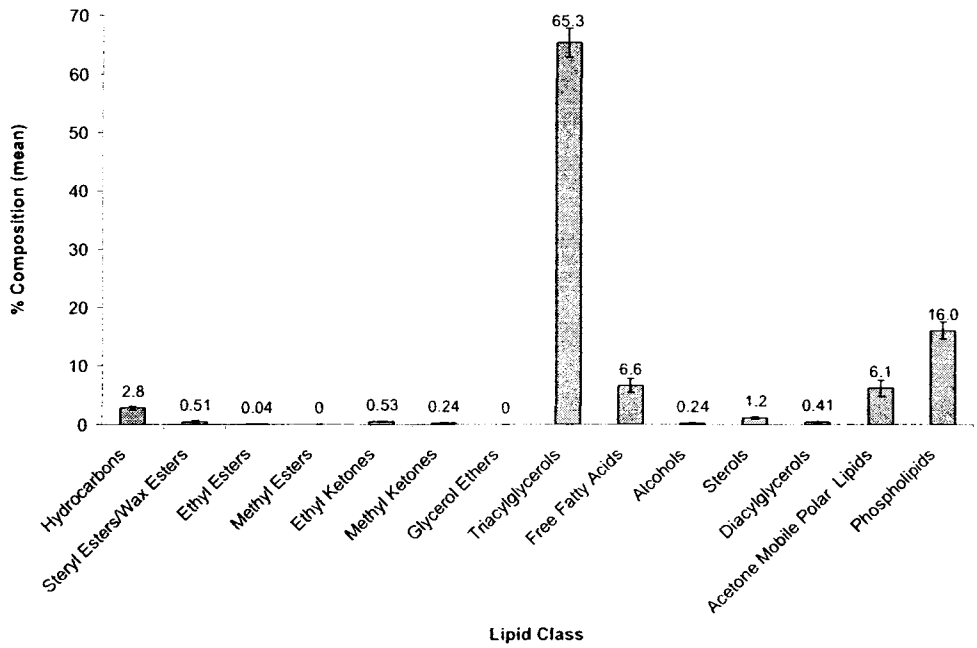

Figure 5.2 Mean % lipid composition for all Hydropsychidae samples tested (n=67). Means are indicated above bars.

5.3.2 Fatty acid composition of hydropsychids

Sixty-five fatty acids were identified and are expressed as a percentage of the total identified fatty acids. One fatty acid (labeled 14:1A) was consistently present but could not be identified by comparison with the standards used. Mass spectrometry analysis showed that it was a fatty acid with a chain length of 14 carbon atoms and one double bond (14:1ω?) but the exact location of the double bond could not be determined.

All eight hydropsychid species had similar fatty acid compositions (Table 5.4). This comprehensive list shows 51 of the 65 identifiable fatty acids were present in low proportions (<1%). The dominant fatty acids (>1%) for all species and life stages were: 14:0, 14:1A, 16:0, 16:1ω9, 16:1ω7, 17:1, 18:0, 18:1ω9, 18:1ω7, 18:2ω6, 18:3ω3, 18:4ω3, 20:4ω6, and 20:5ω3. These 14 fatty acids comprised approximately 89% of the total

identified fatty acid composition in all samples. Twelve of these fatty acids each made up more than 2% of the total fatty acids, with only 14:1A and 16:1ω9 not greater than 2%, and accounted for at least 85.9% of the total fatty acid composition of a species.

The dominance among all species and all life stages of the above 14 fatty acids made them a focus of comparison (Table 5.5). Nine of them (not 14:0, 14:1a, 16:0, 16:1ω9, 18:3ω3) differed among species and three (14:1a, 16:1ω9, 16:1ω7) differed among life stages and so life stages were considered separately among (Table 5.5) and within (Table 5.6) species. Using only larvae, ten of the fatty acids significantly differed among species with most differences primarily caused by *P. apicalis* (high 16:1ω7, 18:2ω6; low 17:1, 18:1ω9, 18:4ω3, 20:4ω6), *D. modesta* (high 16P:1ω7, 18:2ω6, 18:4ω3; low 20:4ω6) and *A. ladogensis* (high 18:0, 20:5ω3; low 14:1a) (Table 5.5). Only four fatty acids significantly differed among species using only pupae, which can partly be attributable to smaller sample sizes. *Parapsyche apicalis* pupae had higher proportions of 18:2ω6 and lower proportions of 17:1 than pupae of the other species, following its larval pattern. *Hydropsyche alternans* pupae had higher proportions of 20:4ω6 than pupae of other species. Differences in proportions of 18:4ω3 among species of pupae were not clearly attributable to one species (Table 5.5). Within a species there were differences between larvae and pupae with, as above, the greatest number of differences evident for *P. apicalis, A. ladogensis* and *H. alternans.* Note that only one pupal sample of *D. modesta* was recovered and no pupae of *H. sparna* were collected (Table 5.6). Further differentiation among species was done with multivariate techniques which included both life stages to increase sample size.

Table 5.4 Mean and standard deviation (+/-) (expressed as percentages of the total identified fatty acids) for all identified fatty acids, by species and overall, with larvae and pupae combined.

	Species of Hydropsychidae																	
fatty acid	*C. pettiti*	+/-	*H. betteni*	+/-	*H. sparna*	+/-	*H. slossonae*	+/-	*H. alternans*	+/-	*A. ladogensis*	+/-	*D. modesta*	+/-	*P. apicalis*	+/-	*Overall*	+/-
14:0	7.24	2.8	7.33	3.1	5.75	4.2	7.37	3.5	4.99	3.2	6.38	4.1	6.98	3.9	6.05	1.2	6.72	3.4
14:1a	1.54	0.8	1.66	0.8	1.28	0.7	1.61	0.6	1.24	0.9	1.51	1.1	1.53	1.2	1.33	0.3	1.49	0.8
14:1	0.44	0.2	0.42	0.2	0.49	0.3	0.44	0.3	0.27	0.2	0.32	0.1	0.35	0.2	0.16	0.1	0.40	0.2
15:0i	0.53	0.5	0.57	0.7	0.31	0.3	0.39	0.3	0.24	0.1	0.24	0.2	0.26	0.2	0.28	0.1	0.41	0.4
15:0ai	0.34	0.2	0.26	0.3	0.34	0.3	0.30	0.2	0.28	0.2	0.20	0.1	0.13	0.1	0.14	0.1	0.28	0.2
15:0	0.41	0.3	0.59	0.5	0.47	0.4	0.47	0.4	0.28	0.2	0.53	0.3	0.34	0.3	0.35	0.1	0.45	0.4
15:1	0.25	0.3	0.25	0.2	0.15	0.2	0.18	0.5	0.12	0.1	0.03	0.1	0.11	0.1	0.12	0.1	0.18	0.3
16:0i	0.26	0.2	0.23	0.1	0.22	0.2	0.22	0.1	0.19	0.2	0.20	0.1	0.17	0.1	0.13	0.1	0.22	0.2
16:0ai	0.04	0.1	0.04	0.0	0.09	0.3	0.04	0.1	0.03	0.0	0.03	0.0	0.06	0.1	0.07	0.1	0.05	0.1
16:0	15.13	1.2	14.61	1.9	15.10	1.9	14.93	1.7	14.73	2.1	13.49	1.5	14.29	2.7	15.39	1.5	14.84	1.8
16:1ω11	0.25	0.2	0.17	0.2	0.27	0.3	0.21	0.2	0.13	0.2	0.30	0.1	0.12	0.2	0.08	0.1	0.20	0.2
16:1ω9	1.42	1.6	1.31	1.5	1.82	1.8	1.05	1.4	0.99	1.2	1.09	1.2	0.48	0.2	0.31	0.1	1.20	1.5
16:1ω7	4.37	2.8	4.54	2.6	3.99	2.7	4.87	2.2	4.15	3.1	4.41	2.7	6.29	3.6	8.70	2.6	4.86	2.9
16:1ω5	0.51	0.3	0.43	0.3	0.49	0.4	0.50	0.2	0.45	0.2	0.43	0.2	0.76	0.6	0.65	0.2	0.50	0.3
17:0i	0.51	0.2	0.44	0.1	0.50	0.3	0.48	0.2	0.46	0.2	0.38	0.1	0.35	0.2	0.34	0.1	0.46	0.2
17:0ai	0.64	0.2	0.62	0.4	0.56	0.2	0.64	0.2	0.50	0.3	0.58	0.2	0.60	0.3	0.33	0.2	0.58	0.3
16:2ω4	0.54	0.7	0.49	0.4	0.67	0.9	0.49	0.3	1.01	1.4	0.40	0.3	0.47	0.2	1.62	0.5	0.66	0.7
17:0	0.87	0.3	0.98	0.3	0.96	0.5	0.81	0.2	0.80	0.4	0.99	0.5	1.29	1.3	1.52	0.4	0.96	0.5
16:3ω4	0.15	0.3	0.33	0.6	0.15	0.2	0.13	0.1	0.35	0.5	0.07	0.1	0.09	0.1	0.54	0.3	0.22	0.4
17:1	4.63	2.6	2.47	1.4	3.46	2.3	4.10	2.3	3.15	2.4	2.92	1.2	3.99	3.4	0.94	0.3	3.37	2.4
16:4ω3	0.50	0.3	0.55	0.3	0.35	0.3	0.54	0.3	0.44	0.3	0.62	0.3	0.48	0.3	0.21	0.1	0.47	0.3
16:4ω1	0.16	0.2	0.12	0.2	0.21	0.4	0.11	0.1	0.16	0.2	0.12	0.1	0.16	0.1	0.50	0.2	0.18	0.2
18:0	4.77	1.2	5.65	1.4	5.89	2.5	5.01	1.3	5.95	1.3	6.21	2.0	5.86	4.5	4.89	0.5	5.39	1.8
18:1ω11	0.03	0.1	0.01	0.0	0.01	0.0	0.03	0.1	0.03	0.1	0.00	0.0	0.01	0.0	0.01	0.0	0.02	0.1
18:1ω9	13.72	2.2	15.24	2.1	12.34	1.7	13.51	2.0	12.65	1.4	13.42	2.0	14.52	3.3	11.36	2.2	13.50	2.3
18:1ω7	3.20	1.2	2.82	0.9	3.11	1.3	2.87	1.5	2.97	0.7	4.02	0.8	3.17	1.5	2.09	0.5	2.99	1.2
18:1ω6	0.02	0.1	0.01	0.1	0.16	0.8	0.03	0.2	0.02	0.1	0.00	0.0	0.12	0.3	0.02	0.1	0.04	0.3
18:1ω5	0.58	1.2	0.31	0.2	0.45	0.4	0.40	0.4	0.29	0.2	0.38	0.2	0.24	0.2	0.28	0.2	0.40	0.6
18:2ω6	4.94	1.5	5.49	1.3	5.40	1.8	5.35	2.0	4.75	1.3	5.15	1.6	6.52	2.7	11.42	4.0	5.79	2.6
18:2ω4	0.18	0.2	0.18	0.1	0.20	0.3	0.17	0.2	0.15	0.1	0.16	0.2	0.13	0.1	0.23	0.1	0.18	0.2
18:3ω6	0.26	0.2	0.31	0.2	0.36	0.3	0.28	0.2	0.32	0.2	0.16	0.2	0.25	0.2	0.34	0.1	0.29	0.2

Table 5.4 continued

fatty acid	Species of Hydropsychidae																			
	C. pettiti	+/-	*H. betteni*	+/-	*H. sparna*	+/-	*H. slossonae*	+/-	*H. alternans*	+/-	*A. ladogensis*	+/-	*D. modesta*	+/-	*P. apicalis*	+/-	*Overall*	+/-		
19:0	0.15	0.2	0.06	0.1	0.10	0.1	0.14	0.2	0.13	0.2	0.20	0.2	0.06	0.1	0.00	0.0	0.11	0.2		
18:3ω4	0.06	0.1	0.08	0.1	0.04	0.1	0.13	0.4	0.06	0.1	0.02	0.0	0.06	0.1	0.28	0.2	0.09	0.2		
18:3ω3	10.71	4.1	10.02	3.1	13.93	5.1	10.56	4.1	12.53	4.8	11.76	5.0	11.16	3.6	8.75	1.0	11.08	4.2		
18:4ω3	2.59	1.2	2.14	1.0	2.28	1.6	3.05	1.7	2.72	1.7	3.17	2.0	3.37	3.2	1.81	0.4	2.55	1.5		
18:4ω1	0.05	0.1	0.02	0.0	0.03	0.1	0.01	0.1	0.10	0.2	0.01	0.0	0.02	0.0	0.05	0.1	0.03	0.1		
20:0	0.32	0.2	0.41	0.2	0.40	0.3	0.36	0.1	0.48	0.3	0.33	0.2	0.27	0.1	0.28	0.1	0.36	0.2		
18:5ω3	0.05	0.1	0.02	0.0	0.11	0.3	0.02	0.1	0.02	0.1	0.00	0.0	0.01	0.0	0.01	0.0	0.03	0.1		
20:1ω11	0.06	0.1	0.03	0.0	0.05	0.1	0.08	0.1	0.06	0.1	0.06	0.1	0.03	0.0	0.08	0.1	0.06	0.1		
20:1ω9	0.14	0.1	0.12	0.1	0.19	0.3	0.20	0.2	0.24	0.3	0.18	0.1	0.13	0.1	0.05	0.0	0.16	0.2		
20:1ω7	0.05	0.2	0.06	0.1	0.22	0.9	0.05	0.1	0.12	0.3	0.06	0.1	0.11	0.1	0.14	0.3	0.09	0.4		
20:2a	0.01	0.1	0.02	0.1	0.05	0.3	0.01	0.0	0.02	0.1	0.00	0.0	0.00	0.0	0.09	0.3	0.02	0.1		
20:2b	0.02	0.1	0.03	0.1	0.04	0.1	0.04	0.1	0.01	0.0	0.05	0.1	0.05	0.1	0.07	0.2	0.03	0.1		
20:2ω6	0.02	0.0	0.06	0.1	0.03	0.1	0.03	0.1	0.03	0.0	0.03	0.0	0.03	0.0	0.08	0.1	0.04	0.1		
20:3ω6	0.14	0.2	0.08	0.1	0.12	0.3	0.08	0.1	0.07	0.1	0.07	0.1	0.10	0.1	0.54	0.6	0.14	0.3		
21:0	0.00	0.0	0.00	0.0	0.05	0.2	0.01	0.0	0.00	0.0	0.01	0.0	0.00	0.0	0.01	0.0	0.01	0.1		
20:4ω6	3.08	1.3	3.95	1.9	3.26	1.6	3.05	1.1	3.85	1.9	3.69	0.8	2.11	1.0	1.97	0.6	3.24	1.5		
20:3ω3	0.04	0.1	0.06	0.1	0.04	0.1	0.05	0.1	0.07	0.1	0.05	0.1	0.05	0.1	0.01	0.0	0.05	0.1		
20:4ω3	0.45	0.3	0.31	0.2	0.27	0.3	0.43	0.4	0.28	0.2	0.29	0.2	1.11	1.6	0.09	0.0	0.36	0.4		
20:5ω3	12.09	2.7	12.05	2.5	11.39	2.9	12.04	2.5	14.92	4.6	13.36	2.0	10.20	2.4	14.53	2.2	12.42	3.0		
22:0	0.40	0.2	0.40	0.3	0.52	0.5	0.35	0.2	0.45	0.2	0.42	0.3	0.36	0.2	0.21	0.1	0.40	0.3		
22:1ω11(13)	0.04	0.1	0.04	0.1	0.04	0.1	0.04	0.1	0.01	0.0	0.01	0.0	0.00	0.0	0.00	0.0	0.03	0.1		
22:1ω9	0.02	0.1	0.01	0.0	0.03	0.1	0.01	0.0	0.01	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.01	0.1		
22:1ω7	0.01	0.1	0.01	0.0	0.01	0.0	0.01	0.0	0.01	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.01	0.0		
22:2NIMDa	0.00	0.0	0.05	0.2	0.01	0.0	0.00	0.0	0.00	0.0	0.03	0.1	0.00	0.0	0.00	0.0	0.01	0.1		
22:2NIMDb	0.00	0.0	0.03	0.1	0.03	0.1	0.01	0.1	0.00	0.0	0.01	0.0	0.00	0.0	0.00	0.0	0.01	0.1		
21:5ω3	0.06	0.1	0.31	1.1	0.03	0.1	0.11	0.3	0.02	0.0	0.06	0.1	0.02	0.0	0.09	0.1	0.11	0.5		
23:0	0.05	0.2	0.01	0.0	0.02	0.1	0.03	0.1	0.00	0.0	0.06	0.2	0.00	0.0	0.00	0.0	0.02	0.1		
22:4ω6	0.02	0.1	0.05	0.2	0.21	0.9	0.03	0.1	0.13	0.5	0.01	0.0	0.03	0.1	0.01	0.0	0.06	0.4		
22:5ω6	0.14	0.1	0.18	0.2	0.15	0.2	0.35	0.6	0.30	0.3	0.22	0.2	0.10	0.1	0.01	0.0	0.19	0.3		
22:4ω3	0.02	0.1	0.01	0.0	0.04	0.2	0.01	0.0	0.03	0.1	0.02	0.0	0.00	0.0	0.04	0.1	0.02	0.1		
22:5ω3	0.09	0.1	0.08	0.2	0.08	0.2	0.22	0.6	0.09	0.1	0.16	0.2	0.04	0.1	0.31	0.3	0.13	0.3		
24:0	0.02	0.1	0.04	0.1	0.04	0.1	0.07	0.2	0.06	0.1	0.06	0.1	0.02	0.0	0.07	0.0	0.05	0.1		
22:6ω3	0.63	0.5	0.77	0.7	0.51	0.7	0.78	0.8	1.04	0.8	0.78	0.5	0.38	0.4	0.05	0.0	0.65	0.7		
24:1	0.04	0.1	0.05	0.1	0.15	0.6	0.09	0.2	0.09	0.2	0.11	0.3	0.04	0.1	0.00	0.0	0.07	0.3		
n	44		42		31		40		18		13		8		18		214			

Table 5.5 Mean and standard deviation (+/-) for the 14 dominant fatty acids by species and life stage. * represent data not being available. p values are given for the difference among species by life stage.

Species	Life Stage	n	14:0	+/-	14:1a	+/-	16:0	+/-	16:1ω9	+/-	16:1ω7	+/-	17:1	+/-	18:0	+/-
C. pettiti	larvae	33	7.25	2.9	1.45	0.9	15.1	1.3	1.56	1.7	4.20	2.9	4.41	2.6	4.81	1.3
	pupae	11	7.20	2.6	1.80	0.7	15.1	1.2	1.01	1.1	4.90	2.7	5.29	2.7	4.66	1.2
H. betteni	larvae	39	7.33	3.0	1.68	0.8	14.7	1.8	1.37	1.6	4.44	2.6	2.41	1.3	5.66	1.4
	pupae	3	7.33	5.0	1.38	0.8	12.9	2.8	0.57	0.1	5.89	2.1	3.18	2.3	5.49	2.6
H. sparna	larvae	31	5.75	4.2	1.28	0.7	15.1	1.9	1.82	1.8	3.99	2.7	3.46	2.3	5.89	2.5
	pupae	0	*	*	*	*	*	*	*	*	*	*	*	*	*	*
H. slossonae	larvae	37	7.32	3.6	1.60	0.6	14.9	1.7	1.10	1.4	4.73	2.1	4.20	2.4	5.05	1.3
	pupae	3	8.02	2.8	1.75	0.3	15.3	1.9	0.48	0.2	6.70	2.2	2.81	1.2	4.55	0.4
H. alternans	larvae	16	4.75	3.3	1.09	0.6	14.8	2.1	1.06	1.2	3.64	2.4	3.32	2.5	6.00	1.3
	pupae	2	6.93	1.2	2.39	2.1	14.1	2.4	0.41	0.2	8.27	6.2	1.74	0.4	5.57	1.7
A. ladogensis	larvae	7	4.63	5.0	0.76	0.7	13.2	1.9	1.68	1.4	2.59	2.3	3.19	1.6	7.06	2.3
	pupae	6	8.42	1.1	2.38	0.6	13.9	0.7	0.41	0.1	6.53	0.9	2.61	0.6	5.22	0.7
D. modesta	larvae	7	6.92	4.2	1.27	1.0	14.2	2.9	0.47	0.3	6.39	3.8	3.83	3.7	5.95	4.9
	pupae	1	7.44	*	3.34	*	15.2	*	0.48	*	5.52	*	5.10	*	5.19	*
P. apicalis	larvae	13	6.13	1.2	1.29	0.3	15.2	1.4	0.31	0.1	8.97	2.8	0.99	0.3	4.91	0.4
	pupae	5	5.85	1.4	1.45	0.2	15.8	1.7	0.31	0.1	8.02	1.8	0.81	0.3	4.86	0.6
pvalues among species(larvae)		183			0.014						<0.0001		<0.0001		0.022	
pvalues among species(pupae)		31											0.007			

Table 5.5 continued

Species	Life Stage	n	18:1ω9	+/-	18:1ω7	+/-	18:2ω6	+/-	18:3ω3	+/-	18:4ω3	+/-	20:4ω6	+/-	20:5ω3	+/-
C. pettiti	larvae	33	14.1	2.1	3.28	1.2	4.90	1.4	9.7	3.4	2.63	1.3	3.29	1.4	12.4	2.7
	pupae	11	12.6	2.2	2.96	1.4	5.05	1.7	13.61	4.9	2.49	1.0	2.46	0.8	11.2	2.4
H. betteni	larvae	39	15.2	2.1	2.83	0.9	5.50	1.4	10.13	3.1	2.05	0.8	3.94	1.9	12.0	2.4
	pupae	3	15.2	0.9	2.60	1.2	5.48	0.6	8.7	2.2	3.40	2.3	4.07	2.5	12.2	4.1
H. sparna	larvae	31	12.3	1.7	3.11	1.3	5.40	1.8	13.9	5.1	2.28	1.6	3.26	1.6	11.39	2.9
	pupae	0	*	*	*	*	*	*	*	*	*	*	*	*	*	*
H. slossonae	larvae	37	13.6	2.1	2.87	1.5	5.25	1.9	10.7	4.2	2.93	1.8	3.08	1.2	12.0	2.6
	pupae	3	12.2	0.5	2.92	0.9	6.62	3.3	8.67	3.2	4.52	0.7	2.69	0.7	12.5	2.2
H. alternans	larvae	16	12.9	1.2	2.99	0.8	4.72	1.4	13.1	4.8	2.61	1.5	3.57	1.7	15.4	4.5
	pupae	2	10.6	1.2	2.77	0.6	4.98	0.2	8.14	1.5	3.56	3.8	6.06	3.2	11.0	5.1
A. ladogensis	larvae	7	13.5	2.2	3.94	0.8	5.77	1.7	13.8	6.0	2.49	2.2	4.00	0.9	14.1	1.7
	pupae	6	13.4	2.0	4.12	0.8	4.43	1.3	9.41	2.4	3.97	1.4	3.32	0.6	12.5	2.1
D. modesta	larvae	7	14.8	3.5	3.21	1.6	6.68	2.9	11.1	3.9	3.68	3.3	1.98	1.0	10.0	2.5
	pupae	1	12.9	*	2.91	*	5.38	*	11.80	*	1.23	*	2.98	*	11.7	*
P. apicalis	larvae	13	10.4	1.1	2.16	0.6	11.7	4.5	8.48	0.8	1.95	0.2	1.98	0.7	15.2	1.9
	pupae	5	14.0	2.2	1.93	0.3	10.6	2.8	9.46	1.1	1.42	0.6	1.95	0.6	12.9	2.1
pvalues among species(larvae)		183	<0.0001		0.042		<0.0001		<0.0001				0.001		<0.0001	
pvalues among species(pupae)		31					0.001				0.036		0.009			

Table 5.6 Comparison of the fatty acid composition by life stage for each species, showing p values where significant (α=0.05) relationships exist. *Hydropsyche sparna* was not included because no pupae were collected.

Species	n	14:0	14:1a	16:0	16:1ω9	16:1ω7	17:1	18:0	18:1ω9	18:1ω7	18:2ω6	18:3ω3	18:4ω3	20:4ω6	20:5ω3
C. pettiti	44								0.05			0.006			
H. betteni	42												0.021		
H. slossonae	40														
H. alternans	18		0.041			0.043			0.023						
A. ladogensis	13		0.001		0.05	0.002									
D. modesta	8														
P. apicalis	18								<0.0001			0.047	0.01		0.039

5.3.2.1 Fatty acid composition among species of hydropsychids

The first three components from a Principal Component Analysis (PCA) using the 14 dominant fatty acids listed above explained 67.7% of variance in the data set (Table 5.7). A plot of these first three components (Figure 5.7) showed *P. apicalis* separating from the other taxa on the 3rd component axis, while segregation of any other species was not evident. Higher positive values of 16:1ω7 and 18:2ω6 were associated with the 3rd component, and *P. apicalis* had higher proportions of these fatty acids (Table 5.7) *Parapsyche apicalis* also had a higher proportion of 16:4ω1 and a lower proportion of 17:1 and 20:4ω6 which separates it from the other species. *Diplectrona modesta* also had a lower proportion of 20:4ω6 compared to the other species.

Table 5.7 Results for the first three components from a PCA using the 14 dominant fatty acids and all samples.

Eigenanalysis of the Covariance Matrix

Eigenvalue	28.1	15.6	10.0
Proportion	0.354	0.197	0.126
Cumulative	0.354	0.551	0.677

Variable	PC1	PC2	PC3
14:0	-0.529	-0.634	-0.188
14:1A	-0.053	-0.062	0.053
16:0	-0.105	-0.164	0.110
16:1ω9	0.117	0.047	-0.143
16:1ω7	-0.334	-0.017	0.579
17:1	0.082	-0.488	-0.194
18:0	0.191	0.227	-0.073
18:1ω9	-0.067	0.066	-0.228
18:1ω7	0.051	0.119	-0.019
18:2ω6	0.040	0.219	0.607
18:3ω3	0.713	-0.409	0.177
18:4ω3	-0.120	-0.081	-0.111
20:4ω6	0.028	0.208	-0.154
20:5ω3	0.074	0.511	-0.252

One-way ANOVA: 16:1ω7 versus Species

```
Source    DF       SS     MS     F      P
Species    7   332.27  47.47  6.62  0.000
Error    206  1476.98   7.17
Total    213  1809.25
S = 2.678   R-Sq = 18.36%   R-Sq(adj) = 15.59%
                    Individual 95% CIs For Mean Based on Pooled StDev of 2.678
Level          N   Mean  StDev  -----+---------+---------+---------+----
A. ladogensis  13  4.407  2.662  (------*------)
C. pettiti     44  4.372  2.811     (---*---)
D. modesta      8  6.286  3.573          (--------*---------)
H. alternans   18  4.153  3.113  (-----*-----)
H. betteni     42  4.541  2.605      (---*---)
H. slossonae   40  4.875  2.170       (---*----)
H. sparna      31  3.988  2.734  (----*----)
P. apicalis    18  8.704  2.567                          (------*-----)
                                 -----+---------+---------+---------+----
                                    4.0       6.0       8.0      10.0
```

One-way ANOVA: 16:4w1 versus Species

```
Source    DF       SS      MS     F      P
Species    7   2.2715  0.3245  7.07  0.000
Error    206   9.4518  0.0459
Total    213  11.7234
S = 0.2142   R-Sq = 19.38%   R-Sq(adj) = 16.64%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.2142
Level          N    Mean   StDev    +---------+---------+---------+---------
A. ladogensis  13  0.1241  0.0804    (-------*-------)
C. pettiti     44  0.1650  0.2013            (---*---)
D. modesta      8  0.1568  0.1040    (---------*---------)
H. alternans   18  0.1595  0.1991        (------*-----)
H. betteni     42  0.1199  0.1531        (---*---)
H. slossonae   40  0.1122  0.1397       (---*----)
H. sparna      31  0.2147  0.3800             (----*----).
P. apicalis    18  0.4994  0.2206                                (-----*------)
                                   +---------+---------+---------+---------
                                  0.00      0.15      0.30      0.45
```

One-way ANOVA: 17:1 versus Species

```
Source    DF       SS     MS     F      P
Species    7   238.06  34.01  7.38  0.000
Error    206   948.84   4.61
Total    213  1186.89
S = 2.146   R-Sq = 20.06%   R-Sq(adj) = 17.34%
                    Individual 95% CIs For Mean Based on Pooled StDev of 2.146
Level          N   Mean  StDev    +---------+---------+---------+---------
A. ladogensis  13  2.922  1.197              (------*-------)
C. pettiti     44  4.625  2.633                              (---*---)
D. modesta      8  3.989  3.441                     (---------*---------)
H. alternans   18  3.146  2.380                  (------*------)
H. betteni     42  2.466  1.410                  (---*----)
H. slossonae   40  4.099  2.321                            (---*----)
H. sparna      31  3.461  2.316                       (----*----)
P. apicalis    18  0.940  0.327    (-----*------)
                                   +---------+---------+---------+---------
                                  0.0       1.5       3.0       4.5
```

One-way ANOVA: 20:4w6 versus Species

Source	DF	SS	MS	F	P
Species	7	72.15	10.31	4.89	0.000
Error	206	434.27	2.11		
Total	213	506.42			

S = 1.452 R-Sq = 14.25% R-Sq(adj) = 11.33%

Individual 95% CIs For Mean Based on Pooled StDev of 1.452

Level	N	Mean	StDev	---------+---------+---------+---------+
A. ladogensis	13	3.685	0.828	(-------*-------)
C. pettiti	44	3.085	1.300	(---*---)
D. modesta	8	2.106	1.020	(---------*---------)
H. alternans	18	3.849	1.927	(-----*------)
H. betteni	42	3.948	1.867	(---*----)
H. slossonae	40	3.047	1.148	(---*----)
H. sparna	31	3.259	1.648	(-----*----)
P. apicalis	18	1.970	0.646	(------*-----)
				---------+---------+---------+---------+
				2.0 3.0 4.0 5.0

Some species differentiation was evident using the 14 dominant fatty acids, but all 65 fatty acids were also used to determine if species could be separated further. A forward stepwise discriminate analysis was conducted in SAS, using a highly discriminatory p value of 0.001 to show a high level of species separation. This method selected ten of the 65 identified fatty acids, entered in the following order: 18:2ω6, 16:4ω1 (these two separate out *P. apicalis*), 20:4w3, 20:5ω3, 18:0, 17:0, 18:1ω9, 16:1ω7, 16:1ω5, 14:1a. A PCA of these ten fatty acids (Figure 5.4), with the first three components explaining 60.7% of the variance, showed that only *P. apicalis* separates. Therefore the differences between the other seven species were slight.

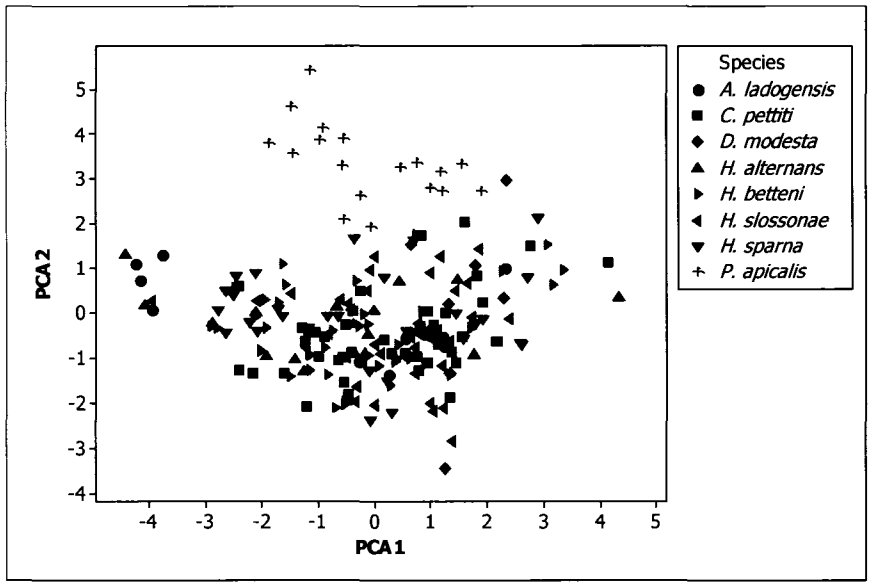

Figure 5.4 A plot of PCA1 versus PCA2 for all samples, larvae and pupae, using the ten fatty acids from the forward stepwise discriminate analysis.

Parapsyche apicalis observations were removed from the data set and the discriminate analysis was run again using the same p value. Only three fatty acids were selected: 18:1ω9, 20:4ω3 and 20:5ω3. *Diplectrona modesta* had significantly ($p<0.0001$) higher levels of 20:4ω3 than the other species, and had lower levels of 20:5ω3. Observations for *D. modesta* were removed from the data set and the discriminate analysis was rerun. Only six fatty acids met the $p=0.001$ criterion: 18:1ω9, 17:1, 18:1ω7, 18:3ω3, 14:1 and 16:4ω3. *Arctopsyche ladogensis* had a significantly higher amount ($p=0.001$) of 18:1ω7 than the other species, and 14:1 was significantly lower ($p<0.0001$) in *A. ladogenis and H. alternans* than the other species. These two species were then removed from the data set, leaving the four most common species (*C. pettiti, H. betteni, H. slossonae, H. sparna*). A forward stepwise discriminate analysis ($p=0.001$) was run on these species resulting in four fatty acids selected: 18:1ω9, 17:1, 18:3ω3 and 18:1ω7.

Consideration of these four fatty acids using one-way ANOVAs showed that *H. sparna* had higher levels of 18:3ω3 and lower levels of 18:1ω9, and that 18:1ω9 was higher in *H. betteni.*

To test whether life stage affected the results, only larval samples were included (n=145). A PCA of the 14 dominant fatty acids showed the first three components explained 66.9% of the variance. *Parapsyche apicalis* clearly separated on PCA3 (Figure 5.5), caused by higher proportions of 18:2ω6 and 16:1ω7 and lower proportion of 17:1 than the other species. These were the same fatty acids that separated *P. apicalis* when larvae and pupae were included and thus life stage was not affecting species separation. Further species differentiation using only fifth instar larvae was not evident.

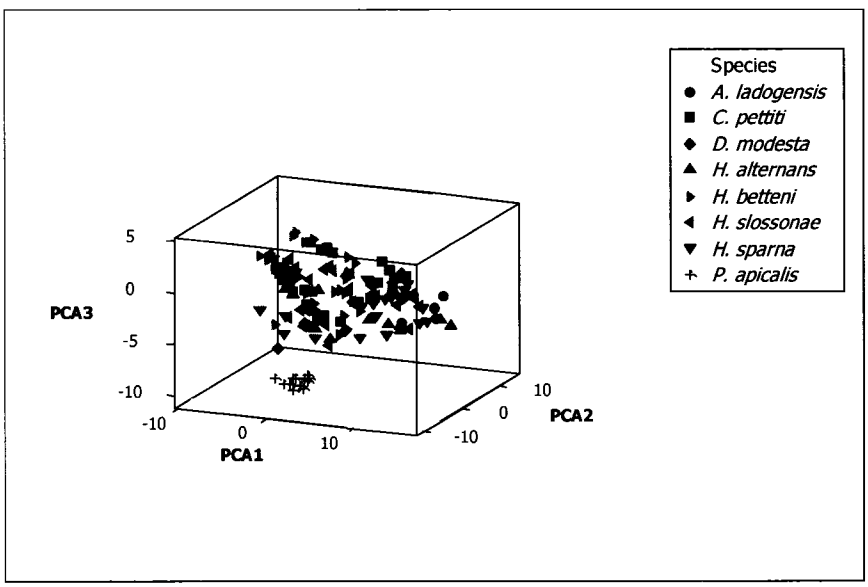

Figure 5.5 Plot of the first three principal components for larvae only using the 14 dominant fatty acids.

Another multivariate technique used to quantify differences amongst species was discriminate function analysis. It was conducted in SAS using all samples, resulting in

82.92% of the samples being correctly identified to species based on the linear discriminate function derived from the 65 fatty acids. This gave an indication of the difference in fatty acid composition between the species, where *P. apicalis* showed the least similarity to the other species (Table 5.9). *Arctopsyche ladogensis* and *D. modesta* showed a distinction from the remaining species but this was much less pronounced than that of *P. apicalis*. *Hydropsyche slossonae* showed the greatest similarity to the other species. Testing was done via resubstitution, where the species' name labels were removed and the samples were reclassified based on the linear discriminate function derived for each species. This resulted in ~83% of the samples being correctly classified, with *A. ladogensis* and *P. apicalis* being correctly identified 100% of the time (Table 5.10). This supports the above PCA results, indicating the fatty acid composition of *A. ladogensis* and *P. apicalis* was distinct from the other species. *Hydropsyche slossonae* was only correctly classified two thirds of the time and was therefore much less distinct (Table 5.10).

Then *P. apicalis* samples were removed from the data set and similarities amongst the species became evident (Table 5.11). The proportion of samples correctly classified using resubstitution declined to ~79%. *Arctopsyche ladogensis, D. modesta* and *H. alternans* had the greatest distance, respectively, from the remaining species. Consideration of the four species which most frequently occurred (*C. pettiti, H. betteni, H. slossonae, H. sparna*) showed *H. betteni* being differentiated the most from the other species and *H. slossonae* the least (Table 5.12) with the overall proportion correctly classified increasing slightly to ~83%.

Table 5.9 Quantification of the distance between species, using a linear discriminant function derived from 65 fatty acids.

Generalized Squared Distance to Species								
From Species	***A. ladogensis***	***C. pettiti***	***D. modesta***	***H. alternans***	***H. betteni***	***H. slossonae***	***H. sparna***	***P. apicalis***
A. ladogensis	0	20.3	30.6	22.6	27.1	19.2	24.0	150.0
C. pettiti	20.3	0	18.5	13.0	12.4	5.0	10.8	118.6
D. modesta	30.6	18.5	0	31.3	22.2	20.3	24.3	114.2
H. alternans	22.6	13.0	31.3	0	16.7	9.5	12.7	131.9
H. betteni	27.1	12.4	22.2	16.7	0	9.2	16.2	111.2
H. slossonae	19.2	5.0	20.3	9.5	9.2	0	9.2	117.2
H. sparna	24.0	10.8	24.3	12.7	16.2	9.2	0	118.2
P. apicalis	150.0	118.6	114.2	131.9	111.2	117.2	118.2	0

Table 5.10 Classification summary of the resubstitution of the data into the derived linear discriminant functions.

Number of Observations (Percent Classified into Species below)									
From Species	*A. ladogensis*	*C. pettiti*	*D. modesta*	*H. alternans*	*H. betteni*	*H. slossonae*	*H. sparna*	*P. apicalis*	Total
A. ladogensis	13	0	0	0	0	0	0	0	13
	100	0	0	0	0	0	0	0	
C. pettiti	1	32	1	2	0	6	2	0	44
	2.3	72.7	2.3	4.6	0	13.6	4.6	0	
D. modesta	0	1	6	0	0	1	0	0	8
	0	12.5	75	0	0	12.5	0	0	
H. alternans	1	0	0	15	0	2	0	0	18
	5.6	0	0	83.3	0	11.1	0	0	
H. betteni	0	3	0	0	34	3	2	0	42
	0	7.1	0	0	81.0	7.1	4.8	0	
H. slossonae	1	6	1	1	3	27	1	0	40
	2.5	15	2.5	2.5	7.5	67.5	2.5	0	
H. sparna	0	3	0	1	0	1	26	0	31
	0	9.7	0	3.2	0	3.2	83.9	0	
P. apicalis	0	0	0	0	0	0	0	18	18
	0	0	0	0	0	0	0	100	
Total	16	45	8	19	37	40	31	18	214
	7.5	21.0	3.7	8.9	17.3	18.7	14.5	8.41	
% Correct	100	72.7	75	83.3	81.0	67.5	83.9	100	82.9

Table 5.11 Quantification of the distance between species using 65 fatty acids with *P. apicalis* samples removed.

Generalized Squared Distance to Species							
From Species	*A. ladogensis*	*C. pettiti*	*D. modesta*	*H. alternans*	*H. betteni*	*H. slossonae*	*H. sparna*
A. ladogensis	5.4	23.3	36.3	29.2	31.5	23.8	26.6
C. pettiti	25.7	3.0	24.1	18.7	15.5	8.4	14.4
D. modesta	35.3	20.7	6.4	36.5	24.8	23.3	27.2
H. alternans	29.9	16.9	38.2	4.8	21.4	14.4	17.5
H. betteni	33.9	15.4	28.1	23.1	3.1	12.1	21.3
H. slossonae	26.1	8.2	26.5	16.0	12.0	3.2	14.0
H. sparna	28.3	13.7	29.9	18.6	20.7	13.5	3.7

Table 5.12 Quantification of the distance between species using 65 fatty acids for the four species which commonly occurred.

Generalized Squared Distance to Species				
From Species	*C. pettiti*	*H. betteni*	*H. slossonae*	*H. sparna*
C. pettiti	2.5	17.4	8.7	15.6
H. betteni	17.4	2.6	12.1	20.2
H. slossonae	8.6	12.0	2.7	13.4
H. sparna	14.9	19.6	12.9	3.2

5.3.2.2 Fatty acid composition within each species with respect to season, location, landscape and stream

Variability among species may be masked by variability within each species, caused by collecting the same species from different streams and collecting in two seasons. Therefore these factors were considered for each species individually. PCAs of the 14 dominant fatty acids for each species explored differences between seasons and streams. Score plots, coded by season (spring, summer) and by stream for each species gave an indication of the degree of separation between these factors (Figure 5.6 to Figure 5.14, Figure 5.10). *Parapsyche apicalis* could not be considered by season because it was only collected in the spring.

To determine if food resource utilization differed depending on a species location in streams and on landscape vegetation patterns, PCAs were conducted on the 14 dominant fatty acids. Score plots, coded by location (outlet, downstream) and by landscape (forested, barren), gave an indication of the degree of separation between these factors within a species (Figure 5.6 to Figure 5.13). Note that Figure 5.10 contains plots by location for three species, arranged in this manner because of page limitations. These plots could not be created for all species because of limitations in occurrence, with *A. ladogensis* only collected at downstream sites, *C. pettiti* and *H. alternans* only collected at outlets and *D. modesta* only collected in forested landscapes.

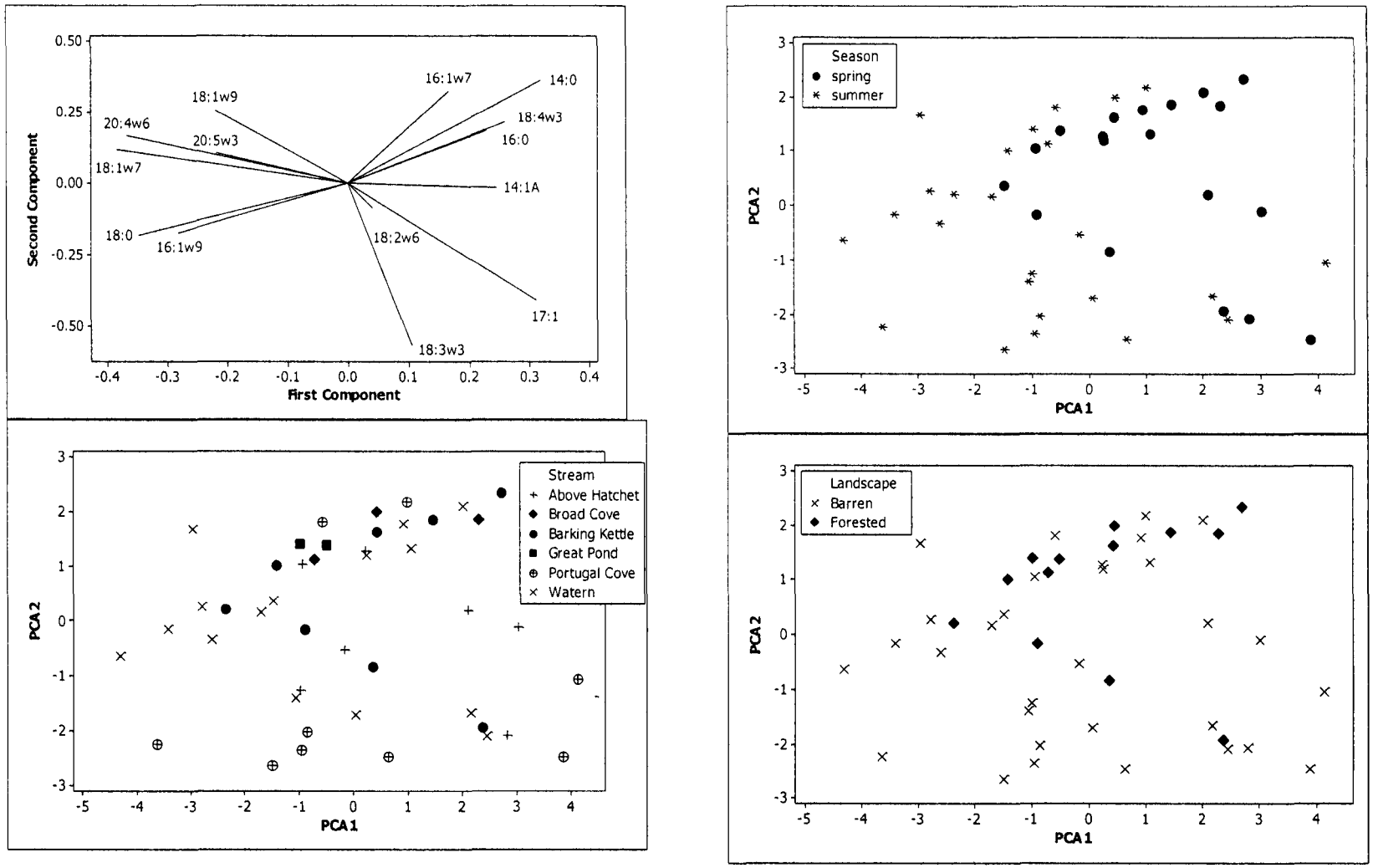

Figure 5.6 All plots are for *Cheumatopsyche pettiti* (n=44). Top left: loading plot of the first and second component. Top right: Score plot of the first two components coded by season. Bottom left: Score plot of the first two components coded by stream. Bottom right: Score plot of the first two components coded by landscape. *Cheumatopsyche pettiti* only occurred at outlets.

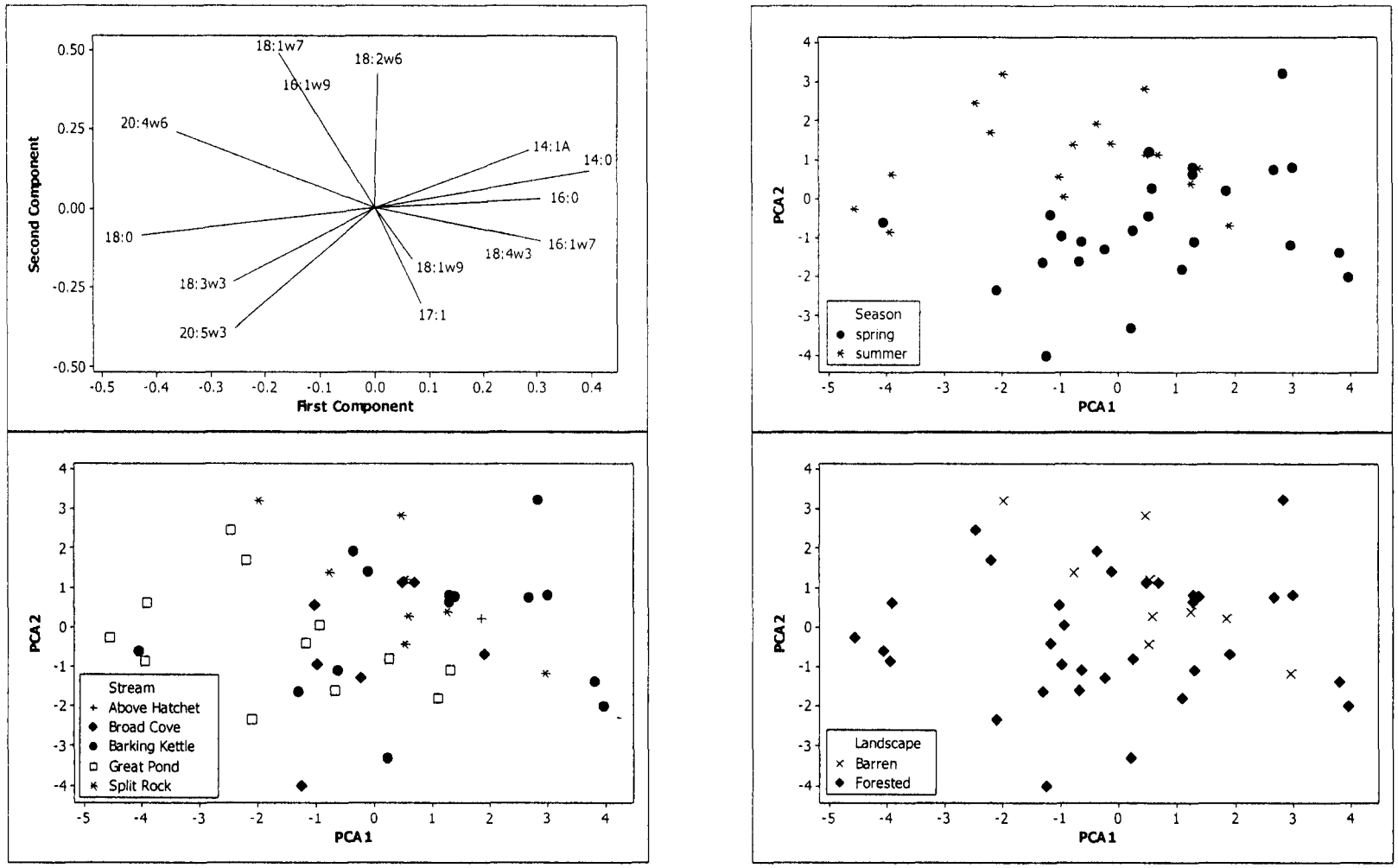

Figure 5.7 All plots are for *Hydropsyche betteni* (n=42). Top left: loading plot of the first and second component. Top right: Score plot of the first two components coded by season. Bottom left: Score plot of the first two components coded by stream. Bottom right: Score plot of the first two components coded by landscape.

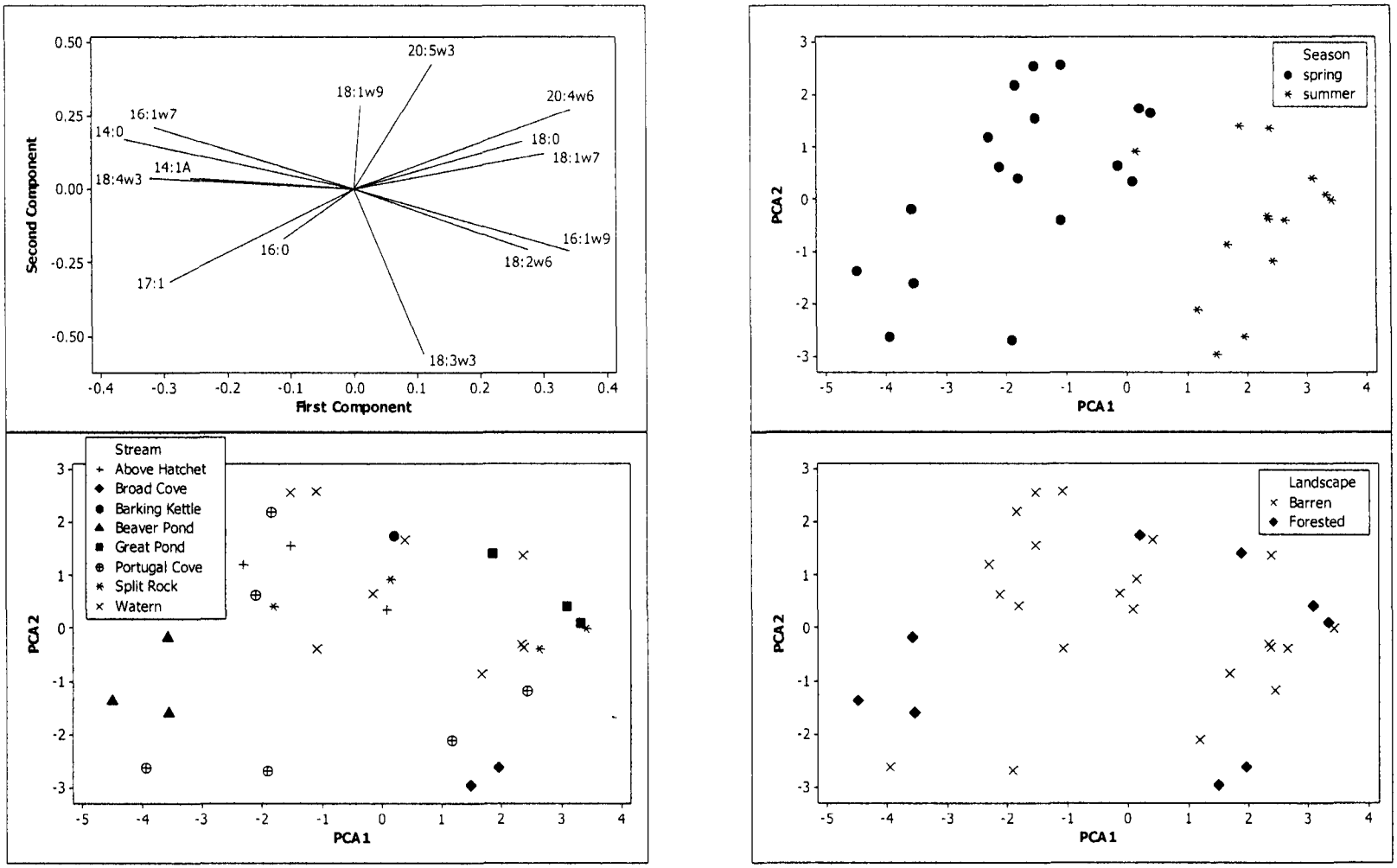

Figure 5.8 All plots are for *Hydropsyche sparna* (n=31). Top left: loading plot of the first and second component. Top right: Score plot of the first two components coded by season. Bottom left: Score plot of the first two components coded by stream. Bottom right: Score plot of the first two components by landscape.

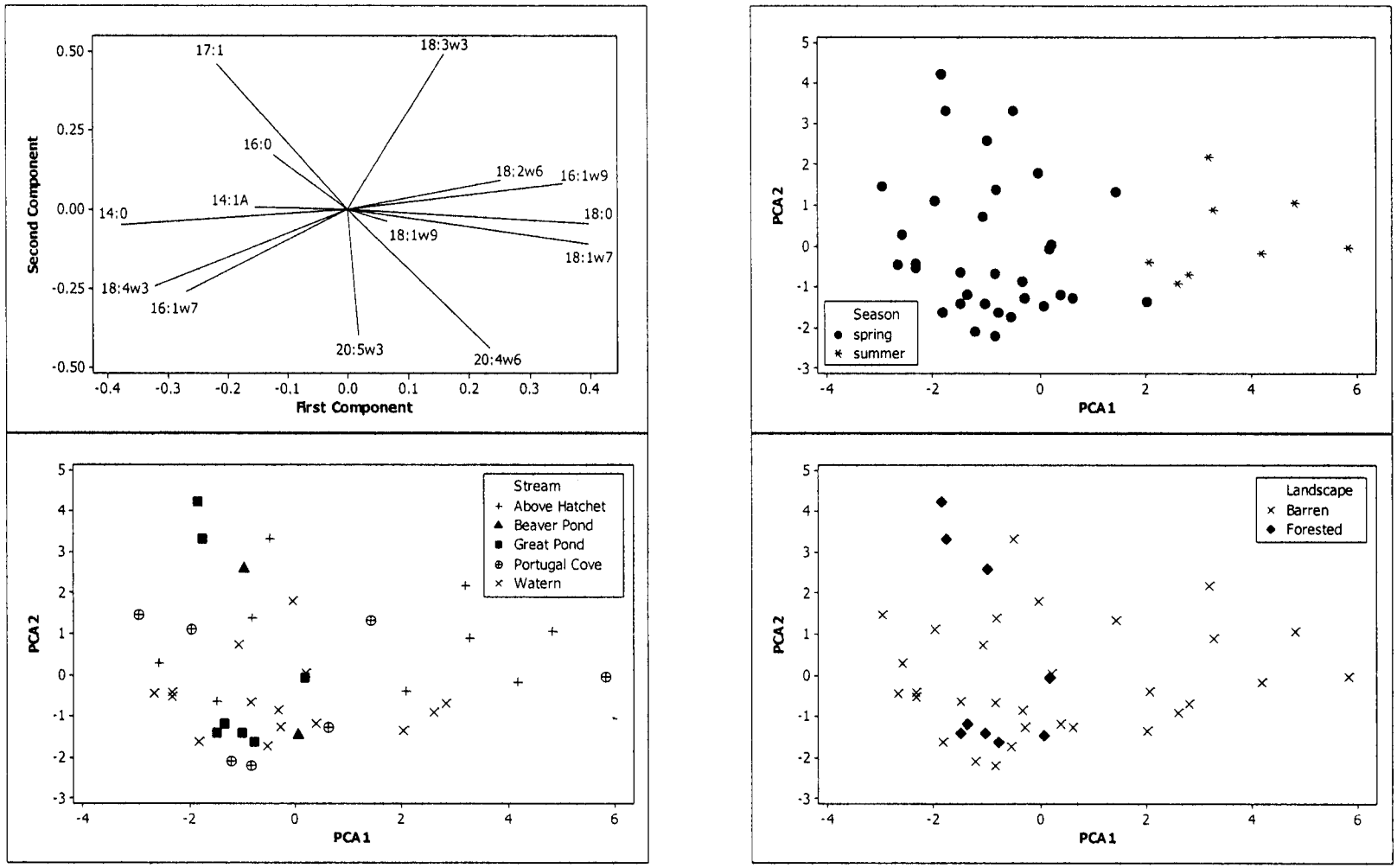

Figure 5.9 All plots are for *Hydropsyche slossonae* (n=40).Top left: loading plot of the first and second component. Top right: Score plot of the first two components coded by season. Bottom left: Score plot of the first two components coded by stream. Bottom right: Score plot of the first two components coded by landscape.

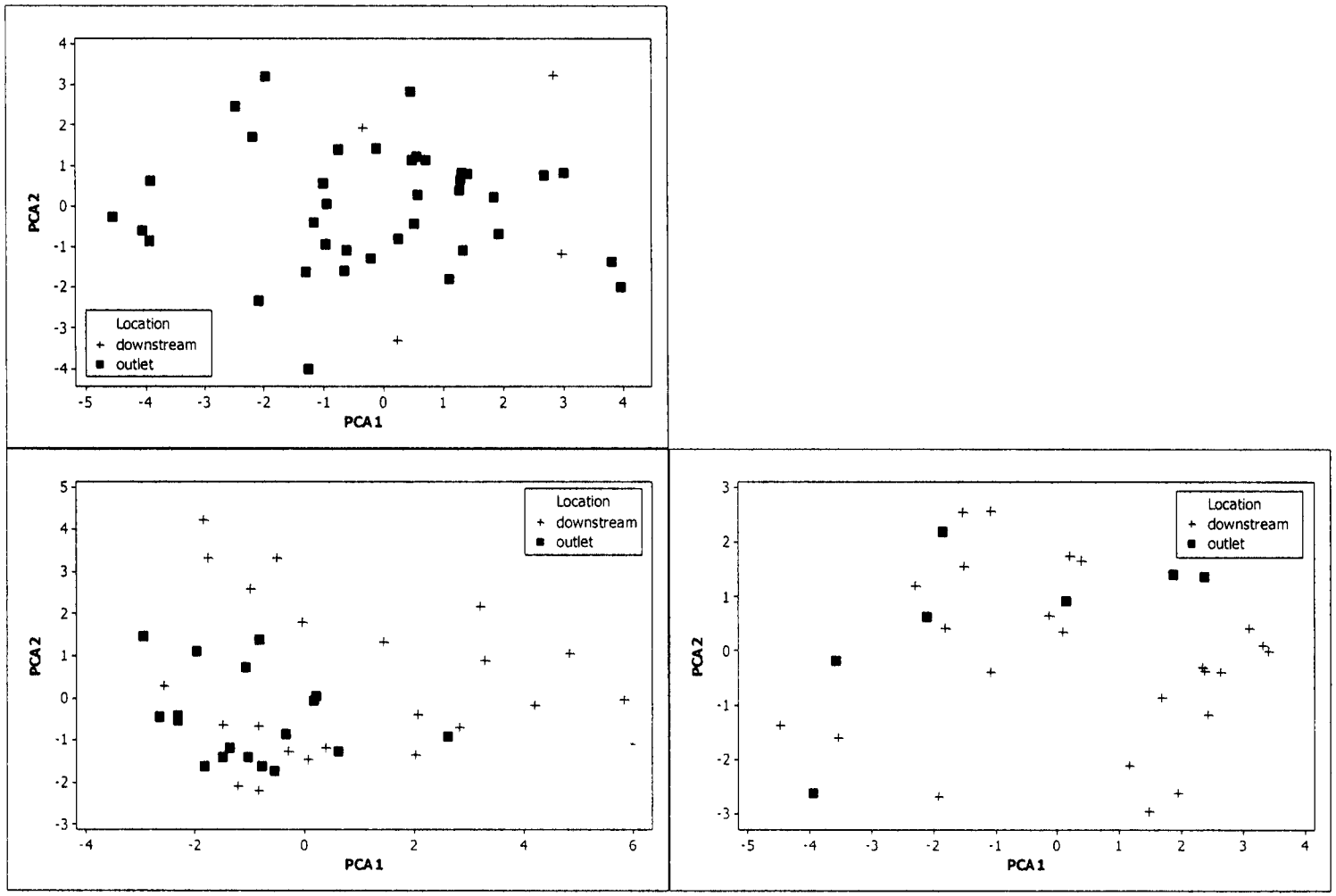

Figure 5.10 All plots are by location. Top left: Score plot of the first two components for *Hydropsyche betteni* (n=42). Bottom left: Score plot of the first two components for *Hydropsyche slossonae* (n=40). Bottom right: Score plot of the first two components for *Hydropsyche sparna* (n=31).

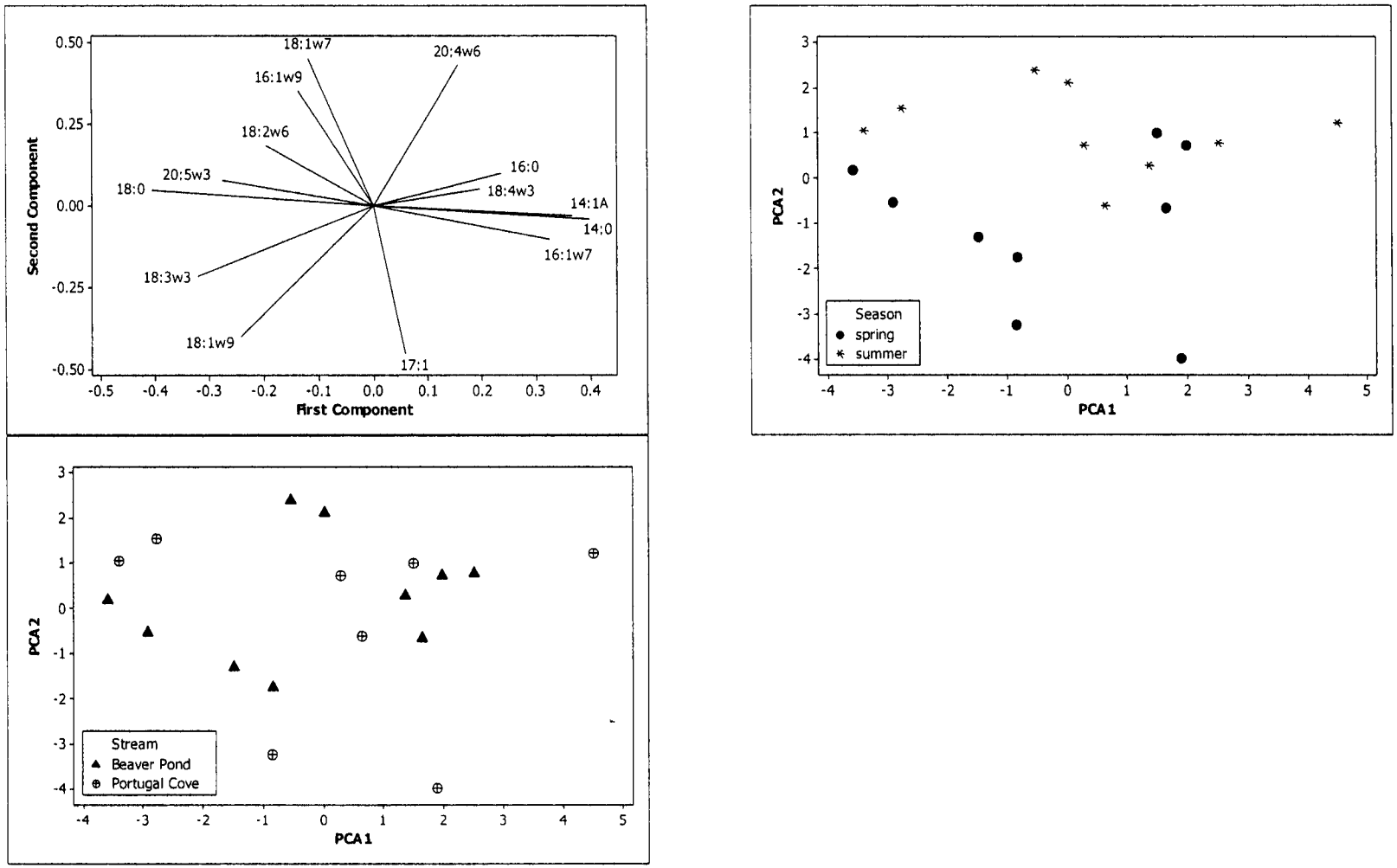

Figure 5.11 All plots are for *Hydropsyche alternans* (n=18).Top left: loading plot of the first and second component. Top right: Score plot of the first two components coded by season. Bottom left: Score plot of the first two components coded by stream. *Hydropsyche alternans* was only collected at outlets. It was also only collected in two streams, so the bottom left figure also shows separation by landscape as Beaver Pond is in a forested landscape and Portugal Cove is in a barren landscape.

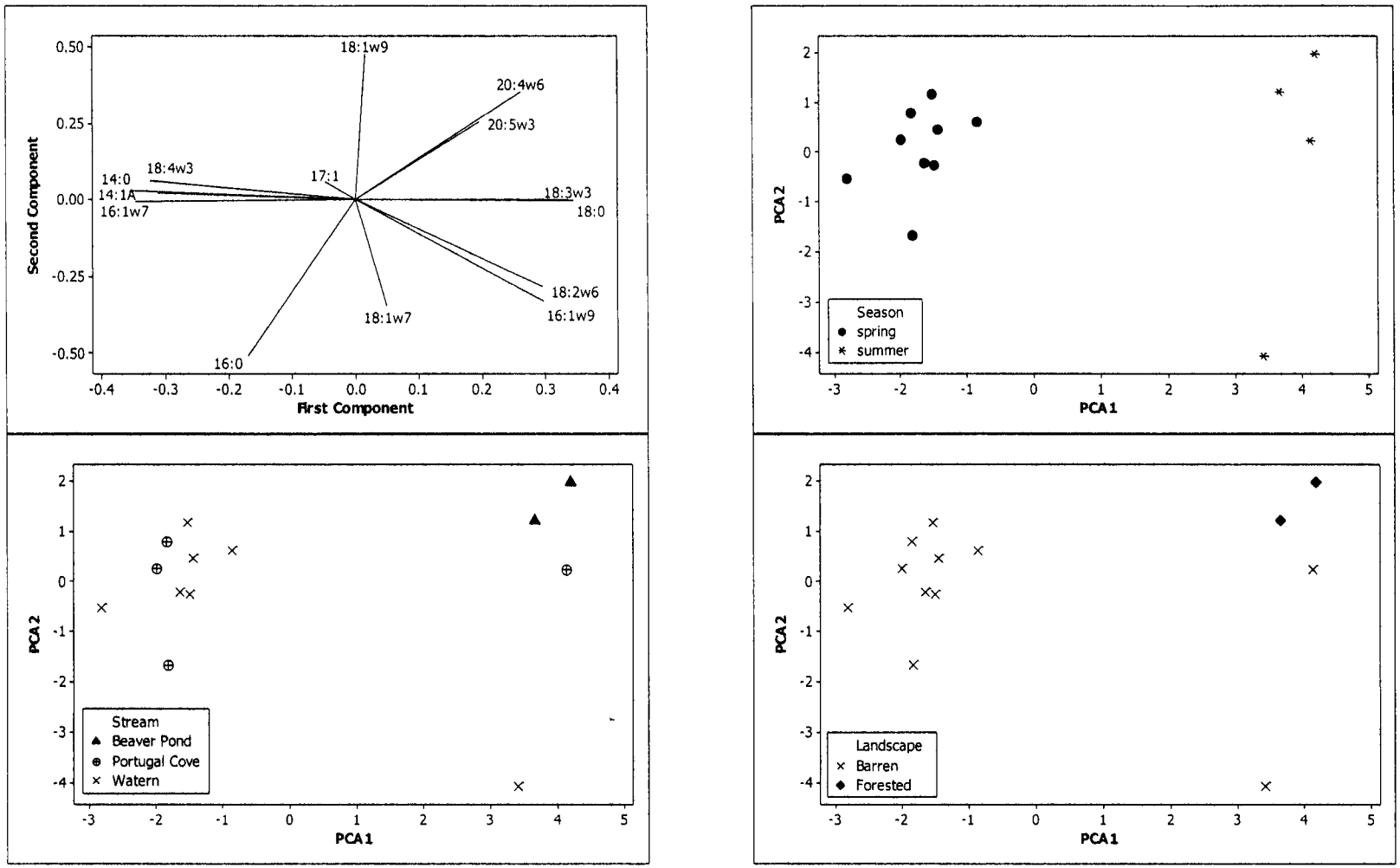

Figure 5.12 All plots are for *Arctopsyche ladogensis* (n=13). Top left: loading plot of the first and second component. Top right: Score plot of the first two components coded by season. Bottom left: Score plot of the first two components coded by stream. Bottom right: Score plot of the first two components coded by landscape. This species did not occur at outlets

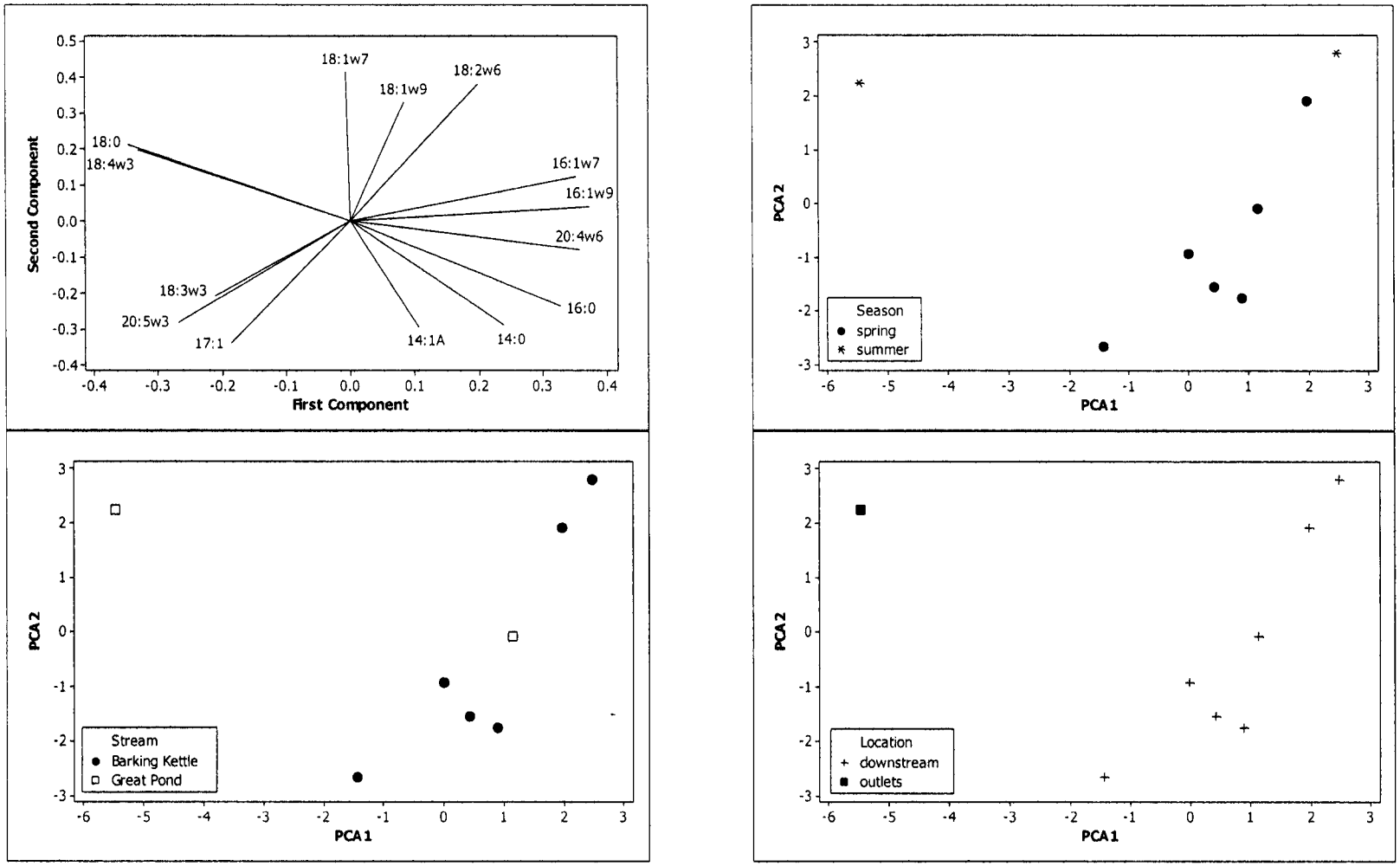

Figure 5.13 All plots are for *Diplectrona modesta* (n=18). Top left: loading plot of the first and second component. Top right: Score plot of the first two components coded by season. Bottom left: Score plot of the first two components coded by stream. Bottom right: Score plot of the first two components coded by location. *Diplectrona modesta* was only collected in forested landscapes.

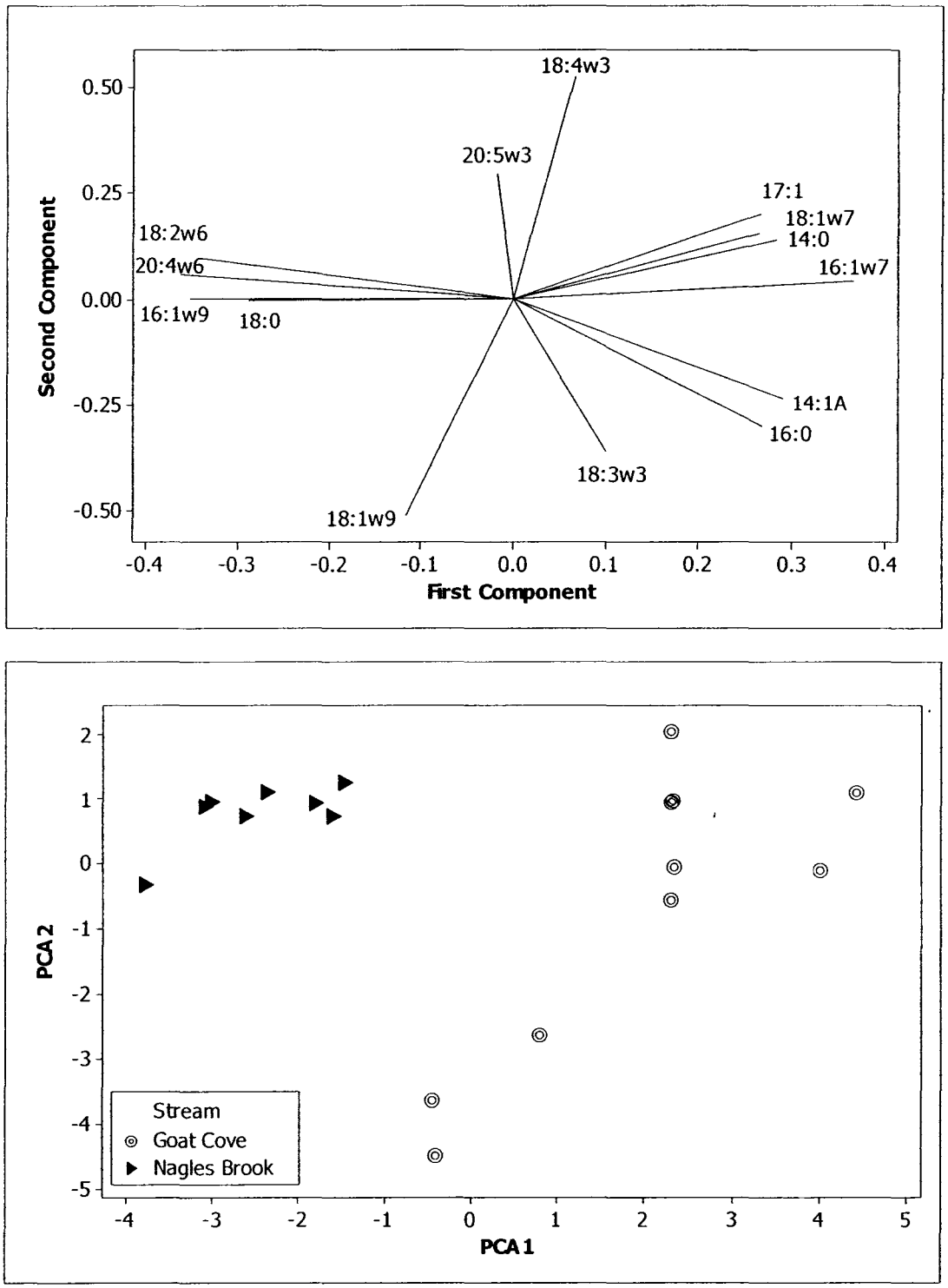

Figure 5.14 All plots are for *Parapsyche apicalis* (n=18). Top: loading plot of the first and second component. Bottom: Score plot of the first two components coded by stream. *Parapsyche apicalis* was only sampled downstream in forested landscapes.

The first two components explained only about half the total variance for most species, with the exception of *A. ladogensis, D. modesta* and *P. apicalis* (Table 5.13). *Cheumatopsyche pettiti* (Figure 5.6) and *H. betteni* (Figure 5.7) did not clearly separate by season, although there was some differentiation. *Hydropsyche alternans* showed no seasonal differentiation (Figure 5.11). *Hydropsyche sparna* (Figure 5.8), *H. slossonae* (Figure 5.11), *A. ladogensis* (Figure 5.12) and *D. modesta* (Figure 5.13) did separate by season, indicating a seasonal shift in food resources across all streams. Comparison of individual fatty acids among seasons for each species quantified these seasonal shifts (Table 5.14). For *H. sparna, H. slossonae* and *A. ladogensis* the proportions of 14:0 and 18:4ω3 were higher in the spring and the proportions of 16:1ω9 and 20:4ω6 were higher in the summer, and so these differing proportions of fatty acids were partly causing the seasonal shift.

The only species which showed a clear differentiation between streams was *P. apicalis* (Figure 5.14), with ten of the 14 fatty acids showing significant differences by stream. The fatty acid composition of *P. apicalis* was much less variable than the other species because it was only collected in the spring in two streams (Table 5.15). The lack of differentiation among streams for species which occurred in many streams indicates that resource use is not restricted within a stream or there would be clear distinctions between streams.

With the exception of the one *D. modesta* collection at an outlet, no species showed a clear differentiation by location or landscape, so these factors were not greatly influencing resource utilization. Comparisons of individual fatty acids for each species by

location (Table 5.16) and by landscape (Table 5.17) demonstrated the lack of significant differences. However, forested samples of *C. pettiti* (Figure 5.6) and *H. slossonae* (Figure 5.9) showed less variance than barren samples. The opposite was true for *H. betteni* (Figure 5.7). *Hydropsyche slossonae* had less variance at outlets compared to downstream samples (Figure 5.9).

Table 5.13 Proportion of the variance explained by the first three principal components (pca1, pca2, pca3) for the 14 dominant fatty acids for each species.

Species	n	pca1	pca2	pca3
C. pettiti	44	0.302	0.481	0.608
H. betteni	42	0.311	0.501	0.644
H. sparna	31	0.391	0.570	0.686
H. slossonae	40	0.335	0.517	0.640
H. alternans	18	0.361	0.570	0.713
A. ladogensis	13	0.527	0.693	0.811
D. modesta	8	0.451	0.757	0.852
P. apicalis	18	0.491	0.719	0.834

Table 5.14 Table of p values from ANOVA analyses of the 14 dominant fatty acids for each species by season.

Species	separation	14:0	14:1A	16:0	16:1ω9	16:1ω7	17:1	18:0	18:1ω9	18:1ω7	18:2ω6	18:3ω3	18:4ω3	20:4ω6	20:5ω3
C. pettiti	no	<0.0001			<0.0001		0.029	0.006		0.001			0.003	0.014	
H. betteni	no				<0.0001	<0.0001				0.008			0.036	<0.0001	
H. sparna	yes	<0.0001	0.001		<0.0001	<0.0001		0.003		0.002	0.004	0.022	0.001	<0.0001	
H. slossonae	yes	0.032			<0.0001	0.024				<0.0001	0.044		0.003	0.027	
H. alternans	no				0.02				0.008					0.002	
A. ladogensis	yes	<0.0001	<0.0001		<0.0001			<0.0001				<0.0001	<0.0001	0.022	
D. modesta	yes		0.044						0.014						
P. apicalis	NA														

Table 5.15 Table of p values from ANOVA analyses of the 14 dominant fatty acids for each species by stream.

Species	separation	14:0	14:1A	16:0	16:1ω9	16:1ω7	17:1	18:0	18:1ω9	18:1ω7	18:2ω6	18:3ω3	18:4ω3	20:4ω6	20:5ω3
C. pettiti	no			0.002					<0.0001						0.03
H. betteni	no		0.008	0.012				0.004							0.044
H. sparna	no		0.002				<0.0001	0.012		0.038			<0.0001	<0.0001	
H. slossonae	no								0.028		0.046		0.014		
H. alternans	no														
A. ladogensis	no	0.041										0.021			0.046
D. modesta	yes												0.027		
P. apicalis	yes	0.036	<0.0001	<0.0001	<0.0001	<0.0001		0.003		0.017	<0.0001	0.010		<0.0001	

Table 5.16 Table of p values from ANOVA analyses of the 14 dominant fatty acids for each species by location.

Species	separation	14:0	14:1A	16:0	16:1ω9	16:1ω7	17:1	18:0	18:1ω9	18:1ω7	18:2ω6	18:3ω3	18:4ω3	20:4ω6	20:5ω3
C. pettiti	NA														
H. betteni	no						0.016				0.008				0.032
H. sparna	no								0.02		0.013				
H. slossonae	no	0.023						0.014		0.005		0.004	<0.0001		
H. alternans	NA														
A. ladogensis	NA														
D. modesta	yes	0.045		0.002	0.014	0.048		<0.0001					<0.0001	0.01	
P. apicalis	NA														

Table 5.17 Table of p values from ANOVA analyses of the 14 dominant fatty acids for each species by landscape.

Species	separation	14:0	14:1A	16:0	16:1ω9	16:1ω7	17:1	18:0	18:1ω9	18:1ω7	18:2ω6	18:3ω3	18:4ω3	20:4ω6	20:5ω3
C. pettiti	no			0.005				0.044	<0.0001						
H. betteni	no														
H. sparna	no			0.031											
H. slossonae	no								0.004	0.006	0.037				
H. alternans	no														
A. ladogensis	no	0.024		0.018		0.019		0.016				0.01	0.038	0.036	
D. modesta	NA														
P. apicalis	NA														

5.3.2.3 Fatty acid composition among species withing a site

A question asked here was whether the coexistence reported in the literature could be explained by partitioning of food resources at a given site by multiple species. Differences in food uptake among species would be reflected in the fatty acid composition. To examine the variation in fatty acid composition among species inhabiting a single stream location, samples were analyzed from sites containing multiple species: Watern outlet, Watern downstream and Barking Kettle outlet. The remaining sites had too few samples of multiple species and therefore analyses were not possible. At Watern outlet 15 samples of *C. pettiti* and 9 samples of *H. slossonae* were collected. Looking at only these samples using the 14 dominant fatty acids, there was a weak separation of the two species on PCA1 (Figure 5.16) with the first two components explaining ~58% of the variance. This separation was caused by lesser amounts of 14:0 ($p=0.048$) and 18:4ω3 ($p=0.002$) and higher amounts of 18:0 ($p=0.046$) in *C. pettiti* compared to *H. slossonae* (Figure 5.15). There was no differentiation amongst or within the species by season.

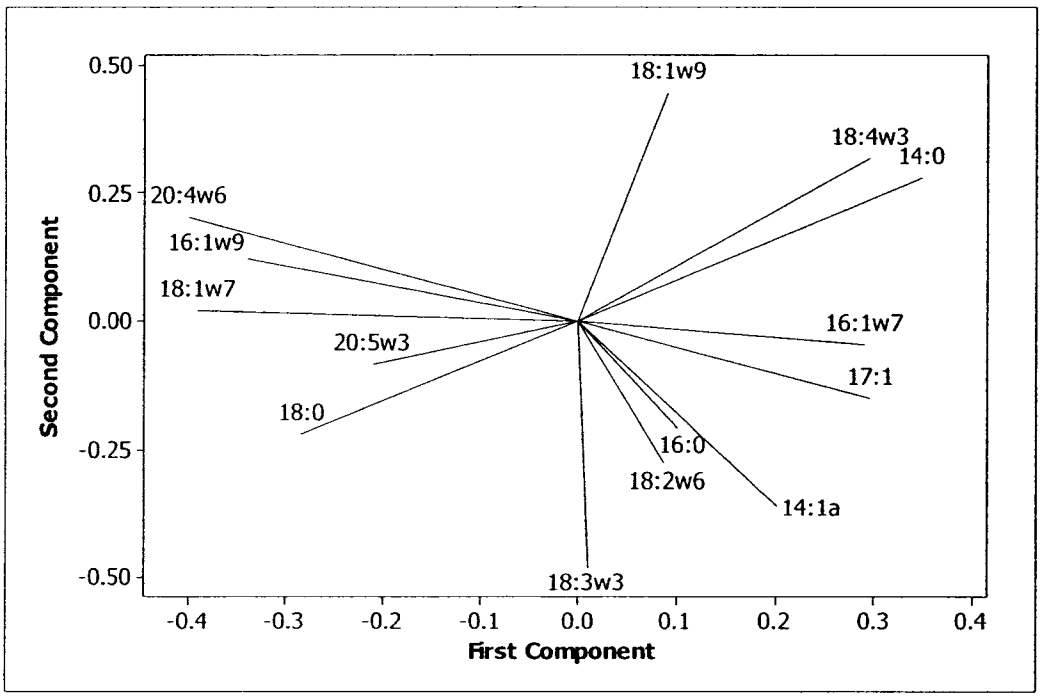

Figure 5.15 Loading plot for the PCA analysis of two species at Watern outlet.

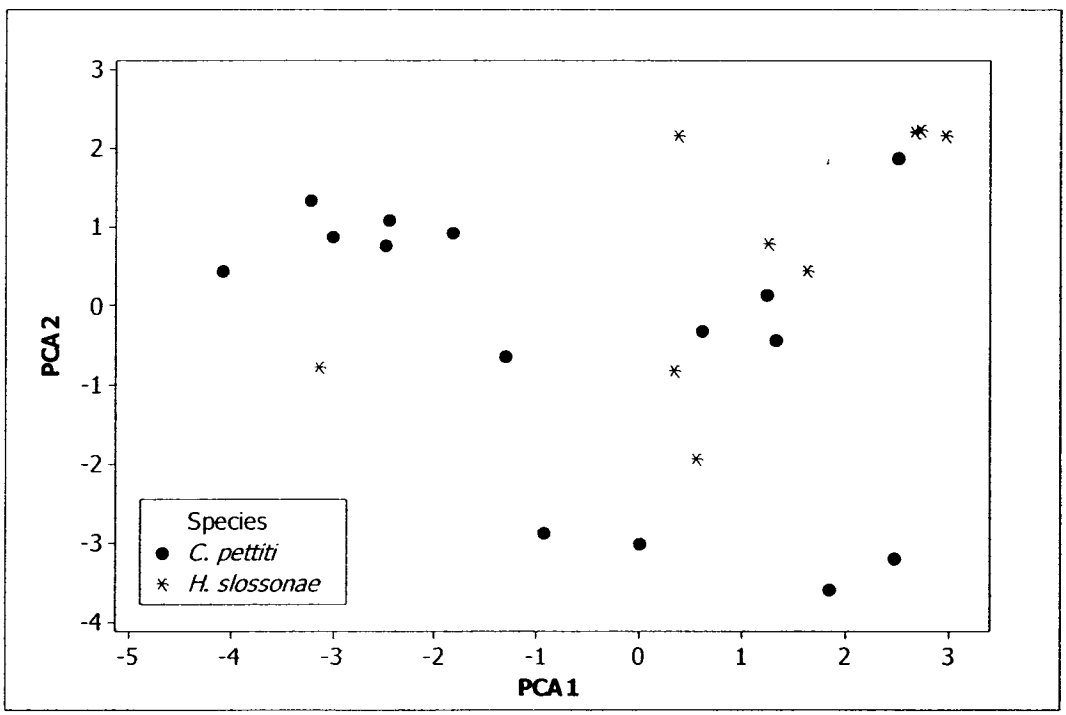

Figure 5.16 Plot of the first two principal components of two species at Watern outlet.

At the downstream location of Watern three species were collected: *A. ladogensis* (n=7), *H. slossonae* (n=6) and *H. sparna* (n=8). The first two components in the PCA analysis explained ~58% of the variance in the data with the loadings plot shown in Figure 5.17. There was no clear distinction amongst the three species (Figure 5.18). *Arctopsyche ladogensis* scored higher on PCA2 but ANOVAs showed no significant differences ($p<0.05$) between the species. Thus the fatty acid composition in terms of the 14 dominant fatty acids does not differ among these three species at the downstream location in Watern. There was some distinction by season for *H. sparna* only with higher levels of 16:1ω9 ($p<0.0001$) and 18:3ω3 ($p=0.039$) and lower levels of 14:0 ($p=0.037$), 16:1ω7 ($p=0.09$) and 18:1ω9 ($p=0.041$) in the summer samples.

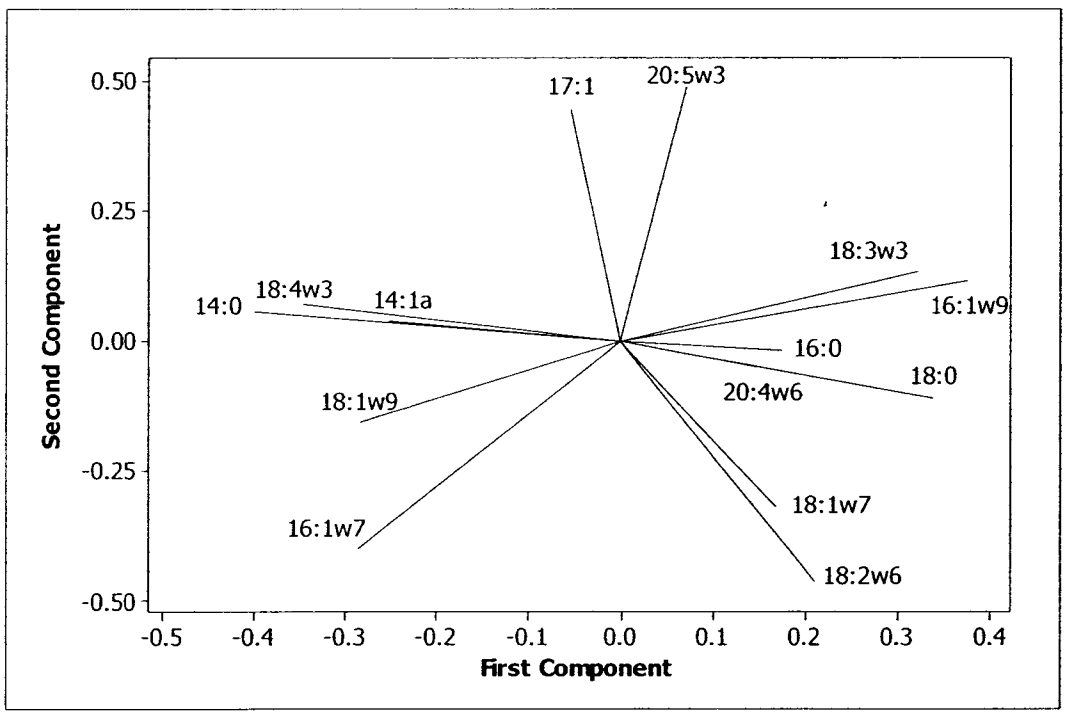

Figure 5.17 Loading plot of the three species located in Watern downstream.

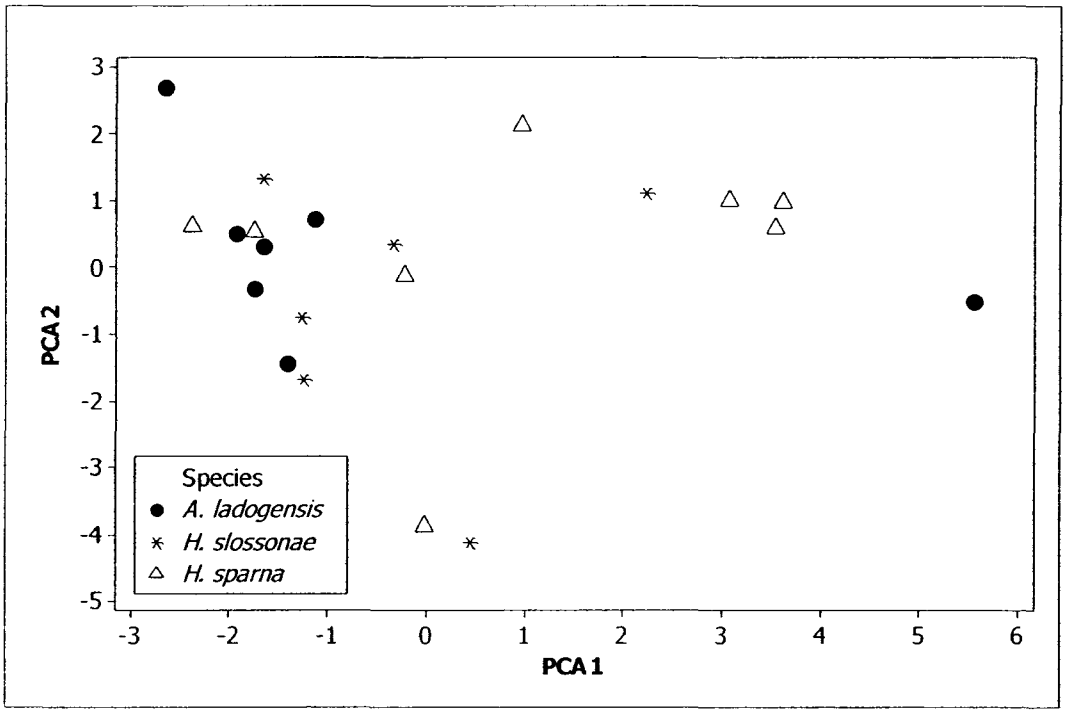

Figure 5.18 Plot of PCA1 versus PCA2 of three species in Watern downstream.

At Barking Kettle outlet *C. pettiti* (n=8) and *H. betteni* (n=11) were collected over both seasons. PCA of the 14 dominant fatty acids explained ~56% of the variance in the first two components (Figure 5.19). The species did not separate (Figure 5.20) based on these fatty acids, nor was there a seasonal separation within or among the two species. There was a site-seasonal difference (combining the two species) with summer samples having elevated levels of 16:1ω9 (p=0.001), 18:1ω9 (p=0.021) and 20:4ω6 (p=0.019) and lower levels of 16:1ω7 (p=0.002). There were no apparent differences by life stage.

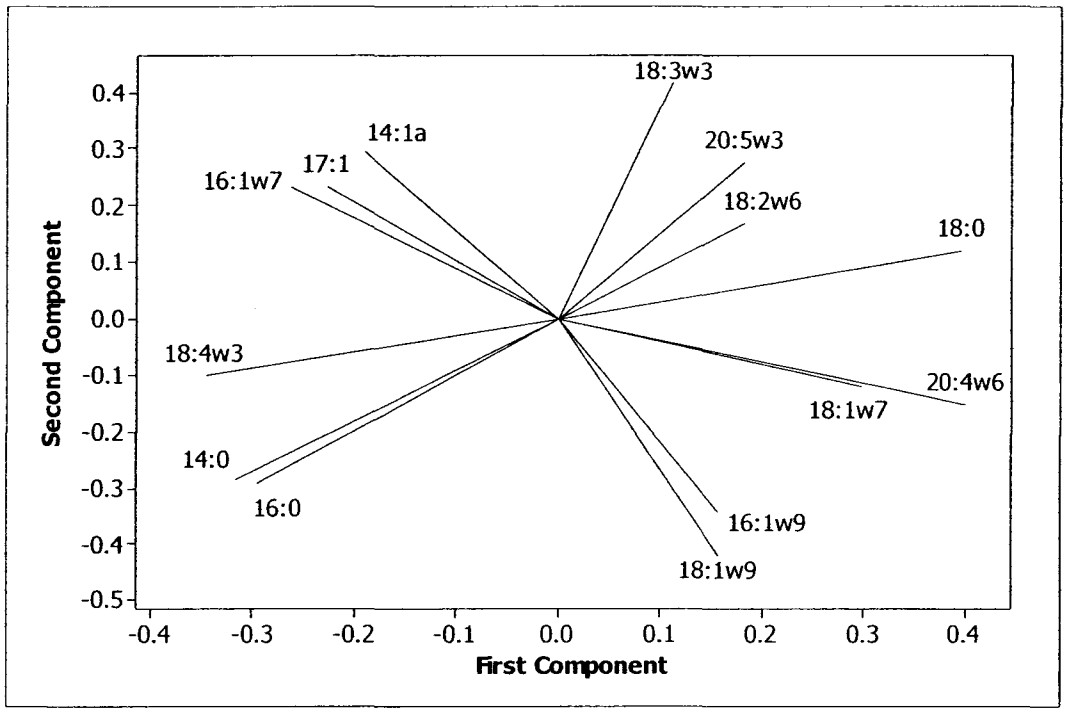

Figure 5.19 Loading plot for the PCA of two species at Barking Kettle outlet.

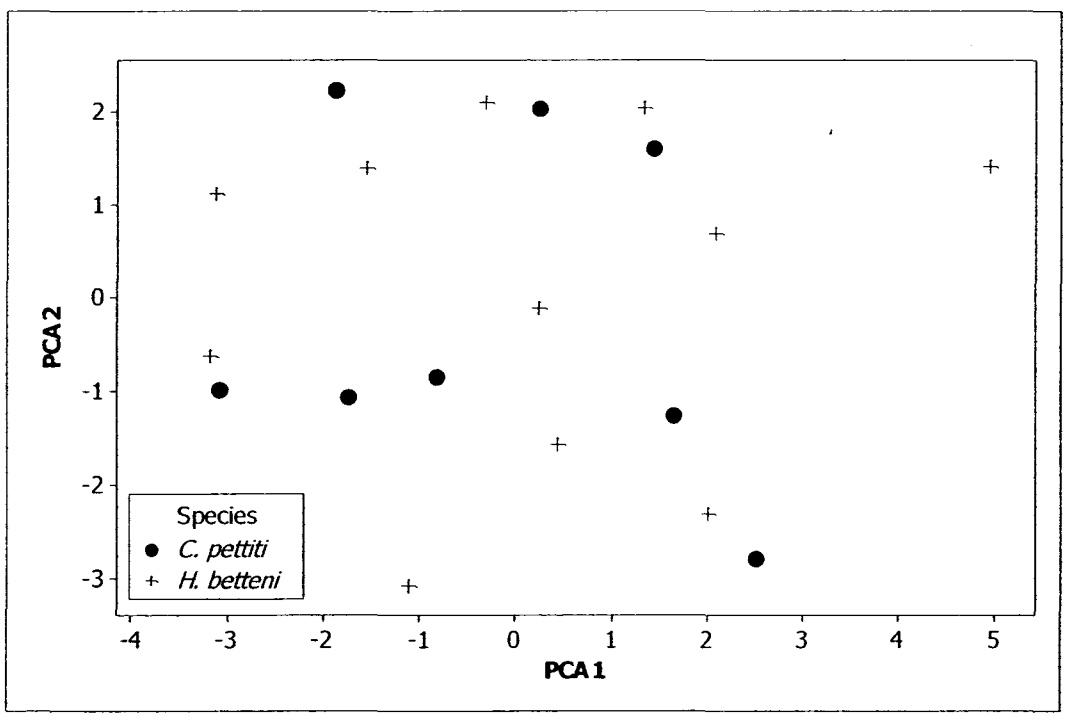

Figure 5.20 Plot of the scores of PCA1 and PCA2 of two species at Barking Kettle outlet.

5.3.3 Fatty acid marker analysis in Hydropsychidae

Differences in fatty acid composition among species and between seasons indicate that species may be utilizing different food resources. Lipid analysis uses fatty acid markers to trace origins of food, such as bacteria, groups of algae or zooplankton. Fatty acid markers used here were derived from the literature with a review given in Appendix 5 (section 10.5). The review includes all potential fatty acid markers, but not all were applicable to this study as discussed below. A summary (the mean and standard deviation) of the fatty acid markers used are given in Table 5.18 for each species and overall.

Fatty acid markers generally differed significantly among species and so analyses were conducted on species separately. For each species, differences by season, location, landscape and life stage were explored. If two or more of these factors significantly differed for a species then, sample size permitting, interactions were examined.

Table 5.18 Mean and standard deviation (+/-) of fatty acid markers by species and over all samples. Formulae for fatty acid markers are given below the table (dinofl.=dinoflagellate).

	Species of Hydropsychidae																	
fatty acid	*C. pettiti*	+/-	*H. betteni*	+/-	*H. sparna*	+/-	*H. slossonae*	+/-	*H. alternans*	+/-	*A. ladogensis*	+/-	*D. modesta*	+/-	*P. apicalis*	+/-	*Overall*	+/-
Σ SAFA	29.35	2.6	30.08	3.3	29.29	3.9	29.58	3.3	27.87	3.3	28.66	2.7	29.47	3.5	28.76	1.7	29.32	3.1
Σ MUFA	29.78	3.2	28.30	2.6	27.43	2.4	28.68	2.7	25.75	3.2	27.74	2.4	30.47	4.9	24.99	2.7	28.10	3.2
Σ PUFA	37.02	4.1	37.78	4.8	39.99	5.0	38.05	3.8	43.44	6.0	40.47	5.4	36.96	4.6	43.63	4.3	39.10	5.1
Σω3 PUFA	27.24	4.3	26.32	4.5	29.03	4.7	27.76	4.0	32.15	5.7	30.26	4.2	26.84	7.5	25.90	2.6	27.88	4.8
ΣC16/ΣC18	0.58	0.1	0.55	0.1	0.54	0.1	0.57	0.1	0.56	0.2	0.49	0.1	0.55	0.2	0.70	0.2	0.57	0.1
Leveille diatoms	0.82	0.3	0.87	0.3	0.71	0.5	0.87	0.3	0.73	0.4	0.83	0.5	0.93	0.4	1.13	0.2	0.85	0.4
$\Sigma C_{18}+C_{22}$ PUFA	3.36	1.5	3.01	1.6	2.98	2.0	4.06	2.3	3.87	2.3	4.11	2.3	3.81	3.1	2.17	0.6	3.37	2.0
Leveille dinofl.	2.89	1.2	2.80	0.9	2.22	1.1	2.93	1.1	2.64	1.1	2.68	1.0	2.45	0.8	2.76	0.3	2.72	1.0
Σω3/Σω6	3.42	1.1	2.86	1.2	3.46	1.6	3.37	1.4	3.75	1.4	3.35	0.6	3.57	2.1	2.10	0.8	3.22	1.3
Golden brown	28.85	2.5	29.86	2.7	27.44	2.4	28.44	2.4	27.37	2.1	26.91	1.6	28.81	4.2	26.75	2.9	28.35	2.7
Bacteria	8.47	2.6	6.46	1.4	7.06	2.3	7.62	2.2	6.06	2.4	6.10	1.3	7.29	3.2	4.22	0.6	6.96	2.4
Terrestrial	15.65	4.6	15.52	3.2	19.33	6.0	15.80	4.9	17.27	5.7	16.91	6.5	17.68	3.6	20.17	3.6	16.85	5.0
Carnivory3	2.02	0.6	2.36	1.0	1.95	0.7	1.86	0.5	2.04	0.7	1.83	0.9	1.81	0.8	1.14	0.4	1.96	0.8
P/S	1.28	0.2	1.28	0.3	1.41	0.3	1.31	0.3	1.60	0.4	1.44	0.3	1.27	0.2	1.53	0.2	1.36	0.3
all essential	31.54	4.5	32.37	4.9	34.57	6.0	32.00	4.6	37.17	6.5	34.90	7.7	30.42	3.4	37.03	4.6	33.32	5.6
essential HUFA	12.81	3.0	12.90	2.7	11.98	3.1	13.03	3.3	16.05	4.8	14.30	1.9	10.63	2.5	14.89	2.1	13.21	3.3
Σ HUFA	16.78	3.7	17.90	4.2	16.13	4.3	17.17	4.3	20.82	5.6	18.72	2.4	14.18	3.4	17.73	2.1	17.42	4.2
n	44		42		31		40		18		13		8		18		214	

where:

Σ SAFA = 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 19:0 + 20:0 + 21:0 + 22:0 + 23:0 + 24:0

Σ MUFA = 14:1 + 15:1 + 16:1ω11 + 16:1ω9 + 16:1ω7 + 16:1ω5 + 17:1 + 18:1ω11 + 18:1ω9 + 18:1ω7 + 18:1ω6 + 18:1ω5 + 20:1ω11 + 20:1ω9 + 20:1ω7+ 22:1ω11(13) + 22:1ω9 + 22:1ω7 + 24:1

Σ PUFA = 16:2ω4 + 16:3ω4 + 16:4ω3 + 16:4ω1 + 18:2ω6 + 18:2ω4 + 18:13ω6 + 18:3ω4 + 18:3ω3 + 18:4ω3 + 18:4ω1 + 18:5ω3 + 20:2α + 20:2β + 20: 2ω6 + 20:3ω6 + 20:4ω6 + 20:3ω3 + 20:4ω3 + 20:5ω3 + 22:NIMDa + 22:NIMDb + 21:5ω3 + 22:4ω6 + 22:5ω6 + 22:5ω3 + 22:6ω3

Σω3 PUFA = 16:4ω3 + 18:3ω3 + 18:4ω3 + 18:5ω3 + 20:3ω3 + 20:4ω3 + 20:5ω3 + 21:5ω3 + 22:5ω3 + 22:6ω3

ΣC16/ΣC18 = (16:0 + 16:1ω11 + 16:1ω9 + 16:1ω7 + 16:1ω5 + 16:2ω4 + 16:3ω4 + 16:4ω3 + 16:4ω1)/(18:0 + 18:1ω11 + 18:1ω9 + 18:1ω7 + 18:1ω6+ 18:1ω5 + 18:2ω6 + 18:2ω4 + 18:13ω6 + 18:3ω4 + 18:3ω3 + 18:4ω3 + 18:4ω1 + 18:5ω3)

Leveille diatoms =(14:0+16:1ω7+16:2ω4+16:3ω4+16:4ω1)/16:0

$\Sigma C_{18}+C_{22}$ PUFA = 18:4ω3+18:5ω3+22:5ω3+22:6ω3

Leveille dinofl. = (16:0+18:4ω3+20:5ω3+22:6ω3) : (18:3ω3+16:2ω4+16:3ω4+16:4ω3+16:4ω1)

Σω3/Σω6 = (16:4ω3 + 18:3ω3 + 18:4ω3 + 18:5ω3 + 20:3ω3 + 20:4ω3 + 20:5ω3 + 21:5ω3 + 22:5ω3 + 22:6ω3) / (18:2ω6 + 18:3ω6 + 20: 2ω6 + 20:3ω6 + 20:4ω6 + 22:4ω6 + 22:5ω6)

Golden brown = 16:0 +18:1ω9

Bacteria = 15:0*i* + 15:0*ai* + 15:0 + 16:0*i* + 16:0*ai* + 17:0*i* + 17:0*ai* + 17:0 + 17:1

Terrestrial = 18:3ω3 + 18:2ω6

Carnivory3 = 18:1ω9 / (16:1ω7 + 18:1ω7)

P/S = Σ PUFA / Σ SAFA

all essential = 18:2ω6 + 18:3ω3 + 20:4ω6 + 20:5ω3 + 22:5ω3 + 22:6ω3

essential HUFA= 20:4ω6 + 20:5ω3 + 22:5ω3 + 22:6ω3

Σ HUFA = 20: 2 ω6 + 20:3 ω6 + 20:4 ω6 + 20:3 ω3 + 20:4ω3 + 20:5 ω3 + 21:5 ω3 + 22:4 ω6 + 22:5 ω6 + 22:5 ω3 + 22:6 ω3

5.3.3.1 Algal fatty acid markers

There were several fatty acid markers for groups of algae, discussed individually below. Algae are very diverse, reflected by the range of fatty acid markers which have been recognized for algal groups (Appendix 5 (section 10.5)). There are general markers of algal fatty acids, as well as those specifically for diatoms, dinoflagellates, green and golden brown algae.

5.3.3.1.1 General algal fatty acid markers

General fatty acid markers of algae are PUFA, particularly ω3 PUFA (Appendix 5 (section 10.5)). All hydropsychids had very high proportions of PUFA (37-44%), with proportions significantly different among species ($p<0.0001$): higher in *H. alternans, A. ladogensis* and *P. apicalis* and lowest in *D. modesta* (Table 5.19). Accounting for 25-30% of the fatty acid composition, ω3 PUFAs followed a similar trend to PUFAs, except for *P. apicalis* which went from having the highest PUFA proportion to the lowest ω3 PUFA proportion. Consideration of individual species by season only gave significant results for *A. ladogensis* with higher amounts of PUFA ($p<0.0001$) and ω3 PUFA ($p=0.001$) in the summer. Other species did not have significant differences by season indicating that algal intake remained constant in spring and summer. By landscape, *A. ladogensis* and *H. slossonae* had higher PUFA ($p=0.08$, $p=0.012$ respectively) and ω3 PUFA ($p=0.022$, $p=0.001$ respectively) content in forested landscapes and *C. pettiti* had higher PUFA ($p=0.012$) content in barren landscapes. There were no significant differences by location, but there were between species which occurred at outlets with *H. alternans* having higher proportions of both markers compared to *C. pettiti* and *H. betteni*

(Table 5.19). Of species more often collected downstream (*A. ladogensis, D. modesta, H. sparna, H. slossonae, P. apicalis*), only *A. ladogensis* and *P. apicalis* had higher proportions, which again indicates a difference among taxa which was not dependent on location (Table 5.19).

Table 5.19 One way ANOVAs of PUFA and ω3 PUFA by species.

One-way ANOVA: PUFA versus Species

```
Source     DF      SS     MS     F      P
Species     7  1101.7  157.4  7.36  0.000
Error     206  4406.2   21.4
Total     213  5508.0
S = 4.625   R-Sq = 20.00%   R-Sq(adj) = 17.28%
                      Individual 95% CIs For Mean Based on Pooled StDev of 4.625
Level          N    Mean  StDev  ----+---------+---------+---------+-----
A. ladogensis  13  40.465  5.386              (-------*------)
C. pettiti     44  37.018  4.090         (---*---)
D. modesta      8  36.959  4.626  (---------*--------)
H. alternans   18  43.441  6.039                        (-----*-----)
H. betteni     42  37.781  4.802           (---*---)
H. slossonae   40  38.053  3.846            (---*---)
H. sparna      31  39.993  4.958                 (---*----)
P. apicalis    18  43.630  4.264                          (-----*-----)
                                  ----+---------+---------+---------+-----
                                   35.0      38.5      42.0      45.5
```

One-way ANOVA: Sum ω3 versus Species

```
Source     DF      SS    MS     F      P
Species     7   643.5  91.9  4.54  0.000
Error     206  4172.1  20.3
Total     213  4815.5
S = 4.500   R-Sq = 13.36%   R-Sq(adj) = 10.42%
                      Individual 95% CIs For Mean Based on Pooled StDev of 4.500
Level          N    Mean  StDev    -+---------+---------+---------+--------
A. ladogensis  13  30.258  4.153                  (-------*-------)
C. pettiti     44  27.235  4.261           (----*---)
D. modesta      8  26.841  7.501    (---------*----------)
H. alternans   18  32.150  5.690                       (------*------)
H. betteni     42  26.317  4.546        (----*---)
H. slossonae   40  27.762  3.997             (----*---)
H. sparna      31  29.032  4.687                (-----*----)
P. apicalis    18  25.902  2.555    (------*------)
                                  -+---------+---------+---------+--------
                                  24.0      27.0      30.0      33.0
```

5.3.3.1.2 Diatom fatty acid markers

All of the diatom fatty acid markers (20:5ω3, 16:4ω1, $\Sigma C_{16}/\Sigma C_{18}$, 16:1/16:0 > 1.6, 16:1ω7/16:0 >1, (14:0 + 16:1ω7 + 16:2ω4 + 16:3ω4 + 16:4ω1)/16:0 = lev. diatoms) (Appendix 5 (section 10.5)) showed significant differences amongst the species, with *P. apicalis* generally having higher amounts. The ratios of 16:1/16:0 and 16:1ω7/16:0 were always less than one, which made them not applicable to this study (Appendix 4). Starting with the most frequently encountered general diatom fatty acid marker in the literature, 20:5ω3, there were significant differences among species with highest amounts in *A. ladogensis, H. alternans* and *P. apicalis* which separated them from the other species (Table 5.20). This agreed with the general PUFA marker for algae above, but results for *P. apicalis* did not agree with the ω3 PUFA marker. *Arctopsyche ladogensis* and *H. alternans* did not maintain their separation from other species when considering other fatty acid markers such as $\Sigma C_{16}/\Sigma C_{18}$ (Table 5.20). This ratio was always below one so there were higher amounts of ΣC_{18} than ΣC_{16} in the hydropsychid samples, with the ΣC_{16} generally indicative of diatoms (Dalsgaard et al. 2003). Therefore a clear pattern of diatom utilization was not evident.

Considering species individually, all but *H. alternans* had higher levels of diatom markers in the spring. *Parapsyche apicalis* was only collected in the spring and could not be compared seasonally (Table 5.20). Species which had higher levels of diatom markers at outlets include: *H. betteni* (20:5ω3 p=0.032), *H. slossonae* (Leveille diatoms p=0.010) and *H. sparna* ($\Sigma C_{16}/\Sigma C_{18}$ p=0.043, 16:4ω1 p=0.009). *Cheumatopsyche pettiti* and *H. alternans* were only collected at outlets and so could not be considered by location. *Hydropsyche alternans* did have significantly higher amounts of 20:5ω3 (p=0.029) than

the other five species sampled at outlets (Table 5.20). Only *H. sparna* showed a significant difference by landscape, with the ratio $\Sigma C_{16}/\Sigma C_{18}$ being higher (p=0.036) in barren landscapes. By life stage, *H. alternans* and *A. ladogensis* pupae had higher proportions of Leveille diatoms (p=0.022, p=0.019 respectively) and $\Sigma C_{16}/\Sigma C_{18}$ (p=0.027, p=0.033 respectively) compared to larval samples. *Parapsyche apicalis* larvae (p=0.039) had higher proportions of 20:5ω3 compared to pupal samples.

Because levels of diatom markers were generally higher in the spring, each season was considered separately and species differences were still significant. Spring trends were the same as mentioned above. In the summer, *A. ladogensis* had much higher levels of 20:5ω3 compared to the other species, but had a lower ratio of $\Sigma C_{16}/\Sigma C_{18}$ (Table 5.20).

Table 5.20 One way ANOVAs for Diatom fatty acid markers

One-way ANOVA: 20:5ω3 versus Species (both seasons)

Source	DF	SS	MS	F	P
Species	7	292.49	41.78	5.41	0.000
Error	206	1592.25	7.73		
Total	213	1884.74			

S = 2.780 R-Sq = 15.52% R-Sq(adj) = 12.65%

Individual 95% CIs For Mean Based on Pooled StDev of 2.780

Level	N	Mean	StDev	---------+---------+---------+---------+
A. ladogensis	13	13.365	1.998	(-------*------)
C. pettiti	44	12.088	2.660	(---*----)
D. modesta	8	10.204	2.385	(---------*---------)
H. alternans	18	14.921	4.635	(------*-----)
H. betteni	42	12.054	2.465	(---*---)
H. slossonae	40	12.039	2.539	(---*----)
H. sparna	31	11.392	2.917	(----*----)
P. apicalis	18	14.528	2.157	(------*-----)
				---------+---------+---------+---------+
				10.0 12.0 14.0 16.0

One-way ANOVA: $\Sigma C_{16}/\Sigma C_{18}$ versus Species (both seasons)

```
Source    DF      SS      MS     F      P
Species    7  0.4156  0.0594  3.20  0.003
Error    206  3.8268  0.0186
Total    213  4.2424
S = 0.1363   R-Sq = 9.80%   R-Sq(adj) = 6.73%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.1363
Level          N    Mean   StDev  --------+---------+---------+---------+-
A. ladogensis  13  0.4946  0.1351  (------*-------)
C. pettiti     44  0.5785  0.1368             (---*---)
D. modesta      8  0.5473  0.1882     (---------*--------)
H. alternans   18  0.5551  0.1909         (------*-----)
H. betteni     42  0.5459  0.1024          (----*---)
H. slossonae   40  0.5717  0.1086            (---*---)
H. sparna      31  0.5423  0.1320         (----*----)
P. apicalis    18  0.6955  0.1752                        (------*-----)
                                  --------+---------+---------+---------+-
                                        0.50      0.60      0.70      0.80
```

One-way ANOVA: 20:5ω3 versus Species (spring)

```
Source    DF      SS     MS     F      P
Species    7  230.94  32.99  3.62  0.001
Error    120 1095.06   9.13
Total    127 1326.00
S = 3.021   R-Sq = 17.42%   R-Sq(adj) = 12.60%
                    Individual 95% CIs For Mean Based on Pooled StDev of 3.021
Level          N    Mean  StDev  --------+---------+---------+---------+-
A. ladogensis   9  12.669  1.727            (-------*-------)
C. pettiti     19  12.637  2.819              (-----*----)
D. modesta      6  10.557  1.724  (---------*---------)
H. alternans    9  16.062  5.966                          (-------*-------)
H. betteni     25  12.376  2.728              (----*---)
H. slossonae   25  12.344  2.776              (---*----)
H. sparna      17  11.245  3.409        (-----*-----)
P. apicalis    18  14.528  2.157                    (-----*-----)
                                 --------+---------+---------+---------+-
                                     10.0      12.5      15.0      17.5
```

One-way ANOVA: 205ω3 versus Species (summer)

```
Source    DF      SS     MS     F      P
Species    6   90.23  15.04  2.74  0.018
Error     79  433.10   5.48
Total     85  523.33
S = 2.341   R-Sq = 17.24%   R-Sq(adj) = 10.96%
                    Individual 95% CIs For Mean Based on Pooled StDev of 2.341
Level          N    Mean  StDev   -+---------+---------+---------+--------
A. ladogensis   4  14.930  1.813                          (-------*-------)
C. pettiti     25  11.670  2.510                    (--*--)
D. modesta      2   9.145  4.688   (----------*----------)
H. alternans    9  13.779  2.669                         (----*----)
H. betteni     17  11.581  2.004                   (---*--)
H. slossonae   15  11.532  2.075                  (---*---)
H. sparna      14  11.570  2.297                  (----*---)
                                  -+---------+---------+---------+--------
                                 6.0       9.0      12.0      15.0
```

One-way ANOVA: 20:5w3 versus Species (outlets only)

```
Source    DF       SS     MS     F      P
Species    5   113.93  22.79  2.60  0.029
Error    120  1051.84   8.77
Total    125  1165.77
S = 2.961   R-Sq = 9.77%   R-Sq(adj) = 6.01%
                Individual 95% CIs For Mean Based on Pooled StDev of 2.961
Level         N    Mean  StDev  --------+---------+---------+---------+-
C pettiti    44  12.087  2.660                (--*--)
D modesta     1  12.462      *  (-------------------*------------------)
H alternans  18  14.920  4.636                          (----*---)
H betteni    38  12.317  2.327                   (--*--)
H slossonae  18  12.421  2.615                 (---*----)
H sparna      7  12.082  3.310              (------*-------)
                                --------+---------+---------+---------+-
                                      9.0      12.0      15.0      18.0
```

One-way ANOVA: $\Sigma C_{16}/\Sigma C_{18}$ versus Species (spring)

```
Source    DF      SS      MS     F      P
Species    7  0.2913  0.0416  3.50  0.002
Error    120  1.4288  0.0119
Total    127  1.7201
S = 0.1091   R-Sq = 16.94%   R-Sq(adj) = 12.09%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.1091
Level           N    Mean   StDev  -----+---------+---------+---------+----
A. ladogensis   9  0.5723  0.0525          (--------*--------)
C. pettiti     19  0.5991  0.0649               (-----*-----)
D. modesta      6  0.6214  0.0847             (----------*----------)
H. alternans    9  0.5080  0.1026  (--------*--------)
H. betteni     25  0.5606  0.1064            (----*----)
H. slossonae   25  0.5959  0.0853               (----*-----)
H. sparna      17  0.6116  0.1232                (-----*------)
P. apicalis    18  0.6955  0.1752                          (-----*-----)
                                  -----+---------+---------+---------+----
                                     0.480     0.560     0.640     0.720
```

One-way ANOVA: $\Sigma C_{16}/\Sigma C_{18}$ versus Species (summer)

```
Source    DF      SS      MS     F      P
Species    6  0.3986  0.0664  2.91  0.013
Error     79  1.8025  0.0228
Total     85  2.2011
S = 0.1511   R-Sq = 18.11%   R-Sq(adj) = 11.89%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.1511
Level           N    Mean   StDev  --+---------+---------+---------+-------
A. ladogensis   4  0.3196  0.0817     (---------*---------)
C. pettiti     25  0.5628  0.1726                          (---*---)
D. modesta      2  0.3253  0.2838  (-------------*-------------)
H. alternans    9  0.6021  0.2489                        (------*------)
H. betteni     17  0.5241  0.0953                     (----*----)
H. slossonae   15  0.5313  0.1326                     (----*-----)
H. sparna      14  0.4582  0.0875                 (-----*----)
                                  --+---------+---------+---------+-------
                                  0.15      0.30      0.45      0.60
```

5.3.3.1.3 Dinoflagellate fatty acid markers

The dinoflagellate fatty acid markers (22:6ω3, $\Sigma C_{18}+C_{22}$PUFA, Leveille dinoflagellate = (16:0 + 18:4ω3 + 20:5ω3 + 22:6ω3)/(18:3ω3 + 16:2ω4 + 16:3ω4 + 16:4ω3 + 16:4ω1), 22:6ω3/20:5ω3, 18:5ω3/18:3ω3) (Appendix 5 (section 10.5)) showed significant differences among species with the exception of Leveille dinoflagellates. All but *H. alternans* had proportions of 22:6ω3 less than 1% (Table 5.21), with proportions in *P. apicalis* being exceptionally low (~0.05%). Proportions of the marker $\Sigma C_{18}+C_{22}$PUFA were less distinct among species, but general trends were similar (Table 5.21). Ratios of 22:6ω3/20:5ω3 and 18:5ω3/18:3ω3 were greatly below one, emphasizing the low proportion of dinoflagellate markers (Table 5.21).

Considering species separately, *C. pettiti* ($p=0.002$), *H. slossonae* ($p=0.005$), *H. sparna* ($p=0.027$) and *A. ladogensis* ($p=0.001$) had significantly higher proportions of $\Sigma C_{18}+C_{22}$PUFA in the spring than other species. *Hydropsyche alternans* had higher proportions of 22:6ω3 in the summer in forested landscapes ($p=0.002$). Only *H. slossonae* showed differences in dinoflagellate markers by location, with outlets having higher proportions of $\Sigma C_{18}+C_{22}$PUFA than downstream samples ($p<0.0001$) and forested outlets having higher proportions than forested downstream samples of 22:6ω3 ($p<0.0001$). There were no differences in 22:6ω3 with location in barren landscapes for this species. There were no significant differences by life stage in individual species.

Table 5.21 One way ANOVAs for dinoflagellate fatty acid markers

One-way ANOVA: 22:6ω3 versus Species

```
Source    DF      SS     MS     F      P
Species    7  11.840  1.691  3.98  0.000
Error    206  87.634  0.425
Total    213  99.473
S = 0.6522   R-Sq = 11.90%   R-Sq(adj) = 8.91%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.6522
Level           N    Mean   StDev  ------+---------+---------+---------+---
A ladogensis   13  0.7754  0.4846                  (--------*--------)
C. pettiti     44  0.6318  0.5248                   (----*----)
D. modesta      8  0.3825  0.3702       (-----------*----------)
H. alternans   18  1.0439  0.8488                            (------*-------)
H. betteni     42  0.7660  0.7320                        (----*----)
H. slossonae   40  0.7760  0.7617                        (----*----)
H. sparna      31  0.5094  0.7106                 (-----*-----)
P. apicalis    18  0.0517  0.0406  (------*-------)
                                  ------+---------+---------+---------+---
                                     0.00      0.40      0.80      1.20
```

One-way ANOVA: $\Sigma C_{18}+C_{22}$PUFA versus Species

```
Source    DF      SS    MS     F      P
Species    7   68.43  9.78  2.68  0.011
Error    206  750.20  3.64
Total    213  818.63
S = 1.908   R-Sq = 8.36%   R-Sq(adj) = 5.24%
                    Individual 95% CIs For Mean Based on Pooled StDev of 1.908
Level           N   Mean  StDev  -------+---------+---------+---------+--
A. ladogensis  13  4.107  2.261                    (---------*---------)
C. pettiti     44  3.360  1.516                 (-----*----)
D. modesta      8  3.814  3.100              (------------*------------)
H. alternans   18  3.867  2.279                   (-------*--------)
H. betteni     42  3.009  1.556              (-----*-----)
H. slossonae   40  4.063  2.310                        (-----*-----)
H. sparna      31  2.977  2.009            (------*------)
P. apicalis    18  2.172  0.591  (--------*--------)
                                 -------+---------+---------+---------+--
                                     2.0       3.0       4.0       5.0
```

One-way ANOVA: 22:6ω3/20:5ω3 versus Species

```
Source    DF       SS        MS     F      P
Species    7  0.06140  0.00877  3.24  0.003
Error    206  0.55708  0.00270
Total    213  0.61849
S = 0.05200   R-Sq = 9.93%   R-Sq(adj) = 6.87%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.05200
Level           N     Mean    StDev  -------+---------+---------+---------+--
A. ladogensis  13  0.06051  0.03889                    (--------*---------)
C. pettiti     44  0.04928  0.03298                    (----*-----)
D. modesta      8  0.03641  0.03304          (-----------*-----------)
H. alternans   18  0.07096  0.05797                          (-------*-------)
H. betteni     42  0.06406  0.05732                          (----*-----)
H. slossonae   40  0.05951  0.05108                        (-----*----)
H. sparna      31  0.04685  0.07975                   (------*-----)
P. apicalis    18  0.00339  0.00260  (-------*-------)
                                    -------+---------+---------+---------+--
                                         0.000     0.030     0.060     0.090
```

5.3.3.1.4 Green algae fatty acid markers

There were several green algae fatty acid markers (18:1ω7/18:1ω9, Leveille green = 16:3ω3 + 18:3ω3 + 20:4ω3, 18:3ω3 + 18:2ω3, ω3/ω6>=2) (Appendix 5 (section 10.5)), but not all were appropriate for this study. The ratio of 18:1ω7/18:1ω9 was always very small (< 0.56) and so this marker was not used. 16:3ω3 was not identified in samples and proportions of 20:4ω3 were very small and so the Leveille marker was not appropriate to use in this analysis. Proportions of 18:3ω3 were high in hydropsychids (8-12%), but 18:2ω3 was not identified and so only 18:3ω3 could be used instead of the summation of these two fatty acids. The ratio of ω3/ω6 was greater than two but less than four in all species (much higher values are indicative of cyanobacteria) and so this ratio and 18:3ω3 were used as fatty acid markers of green algae. Species showed significant differences in their proportions of 18:3ω3 and ω3/ω6 ratio with *P. apicalis* having the lowest proportions and with less distinction amongst the other species (Table 5.22). Considering species separately, seasonal differences were significant for *H. betteni* (p=0.001), *H. sparna* (p=0.012) and *H. alternans* (p=0.005) which had higher proportions of ω3/ω6 in the spring. Proportions of 18:3ω3 were higher in summer samples of *A. ladogensis* (p<0.0001) than in spring samples. By location, only *H. slossonae* had higher proportions of 18:3ω3 (p=0.004) downstream than at outlets. By landscape, only *A. ladogensis* had a higher proportion of 18:3ω3 (p=0.010) in forested than in barren landscapes. Pupal samples of *C. pettiti* (p=0.006) also had higher proportions of 18:3ω3 compared to larval samples. *Hydropsyche sparna* had higher proportions of 18:3ω3 than other species located downstream (Table 5.22).

Table 5.22 One way ANOVAs of Green Algae fatty acid markers

One-way ANOVA: 18:3ω3 versus Species

```
Source     DF      SS     MS     F      P
Species     7   456.7   65.2  4.01  0.000
Error     206  3348.2   16.3
Total     213  3804.9
S = 4.032   R-Sq = 12.00%   R-Sq(adj) = 9.01%
                    Individual 95% CIs For Mean Based on Pooled StDev of 4.032
Level          N    Mean  StDev  --+---------+---------+---------+-------
A. ladogensis  13  11.755  5.050            (--------*--------)
C. pettiti     44  10.713  4.121            (----*----)
D. modesta      8  11.163  3.628       (-----------*-----------)
H. alternans   18  12.527  4.837                 (------*-------)
H. betteni     42  10.024  3.073          (----*----)
H. slossonae   40  10.564  4.090            (----*----)
H. sparna      31  13.932  5.056                      (-----*----)
P. apicalis    18   8.753  0.955  (------*-------)
                                 --+---------+---------+---------+-------
                                 7.5      10.0      12.5      15.0
```

One-way ANOVA: ω3/ω6 versus Species

```
Source     DF      SS     MS     F      P
Species     7   38.84   5.55  3.33  0.002
Error     206  343.25   1.67
Total     213  382.09
S = 1.291   R-Sq = 10.16%   R-Sq(adj) = 7.11%
                    Individual 95% CIs For Mean Based on Pooled StDev of 1.291
Level          N   Mean  StDev   -+---------+---------+---------+--------
A. ladogensis  13  3.348  0.578                 (--------*--------)
C. pettiti     44  3.419  1.112                      (----*----)
D. modesta      8  3.568  2.141                 (-----------*-----------)
H. alternans   18  3.751  1.443                       (-------*------)
H. betteni     42  2.857  1.217               (----*----)
H. slossonae   40  3.368  1.372                     (----*----)
H. sparna      31  3.464  1.571                      (----*-----)
P. apicalis    18  2.099  0.781   (------*-------)
                                 -+---------+---------+---------+--------
                                1.60      2.40      3.20      4.00
```

5.3.3.1.5 Golden brown algae fatty acid markers

Fatty acid markers for golden brown algae included ω3/ω6 >18 and 16:0 + 18:1ω9 (Appendix 5 (section 10.5)). The ratio of ω3/ω6 did not exceed 18 and so this fatty acid marker was not used. The sum of 16:0 + 18:1ω9 was high (~28%) and differed among species (Table 5.23) with *P. apicalis* and *A. ladogensis* having lower proportions and *H. betteni* higher proportions. Considering species separately, only *C. pettiti*

(p<0.0001) showed a significant difference with forested landscapes having higher proportions than barren landscapes.

Table 5.23 One way ANOVA for Golden Brown Algae fatty acid markers

One-way ANOVA: 16:0 + 18:1ω9 versus Species

Source	DF	SS	MS	F	P
Species	7	224.91	32.13	4.87	0.000
Error	206	1358.67	6.60		
Total	213	1583.58			

S = 2.568 R-Sq = 14.20% R-Sq(adj) = 11.29%

Individual 95% CIs For Mean Based on Pooled StDev of2.568

Level	N	Mean	StDev	+---------+---------+---------+---------
A. ladogensis	13	26.905	1.573	(--------*---------)
C. pettiti	44	28.850	2.507	(----*----)
D. modesta	8	28.813	4.204	(-----------*-----------)
H. alternans	18	27.373	2.149	(------*-------)
H. betteni	42	29.857	2.702	(----*----)
H. slossonae	40	28.440	2.446	(-----*----)
H. sparna	31	27.435	2.437	(-----*-----)
P. apicalis	18	26.747	2.928	(-------*-------)
				+---------+---------+---------+---------
				25.5 27.0 28.5 30.0

5.3.3.2 Cyanobacteria and Bacteria fatty acid markers

Fatty acid markers of cyanobacteria were 18:1ω7 and 18:3ω6 (Appendix 5 (section 10.5)). Proportions of 18:1ω7 differed significantly among species (p=0.001), but 18:3ω6 (p=0.098) did not significantly differ among species (Table 5.24). Proportions of 18:1ω7 were significantly higher in the summer for *C. pettiti* (p=0.001) and *H. betteni* (p=0.008) compared to the spring. Considering seasons separately, in the spring *H. slossonae* (p=0.014) had higher proportions downstream compared to outlets and in the summer *H. sparna* (p=0.012) had higher proportions in barren compared to forested landscapes. There were no differences by life stage.

Table 5.24 One way ANOVA of Cyanobacteria fatty acid markers

One-way ANOVA: 18:1ω7 versus Species

Source	DF	SS	MS	F	P
Species	7	32.77	4.68	3.62	0.001
Error	206	266.13	1.29		
Total	213	298.90			

S = 1.137 R-Sq = 10.96% R-Sq(adj) = 7.94%

Individual 95% CIs For Mean Based on Pooled StDev of 1.137

Level	N	Mean	StDev	+---------+---------+---------+---------
A. ladogensis	13	4.022	0.762	(------*-------)
C. pettiti	44	3.199	1.234	(---*---)
D. modesta	8	3.174	1.461	(---------*---------)
H. alternans	18	2.964	0.732	(------*------)
H. betteni	42	2.816	0.872	(---*----)
H. slossonae	40	2.870	1.461	(----*---)
H. sparna	31	3.109	1.301	(----*----)
P. apicalis	18	2.094	0.512	(-----*------)
				+---------+---------+---------+---------
				1.60 2.40 3.20 4.00

The bacteria fatty acid marker was the sum of 15:0*i*, 15:0*ai*, 15:0, 16:0*i*, 16:0*ai*, 17:0*i*, 17:0*ai*, 17:0 and 17:1 (Appendix 4). This marker accounted for ~4-9% of the total fatty acids identified, and differed significantly ($p<0.0001$) among species, with *C. pettiti* showing the highest proportion of bacteria compared to the other species (Table 5.25). Amounts of the bacteria fatty acid marker in *H. slossonae, H. sparna* and *D. modesta* were very similar (~7%), as were those of *A. ladogensis, H. alternans* and *H. betteni* (~6%). Consideration of species individually showed there were no significant differences in fatty acid bacteria makers by season, location, landscape or life stage.

Table 5.25 One way ANOVAs of bacteria fatty acid markers

One-way ANOVA: Bacteria versus Species

Source	DF	SS	MS	F	P
Species	7	288.96	41.28	9.23	0.000
Error	206	921.30	4.47		
Total	213	1210.27			

S = 2.115 R-Sq = 23.88% R-Sq(adj) = 21.29%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev of 2.115
A. ladogensis	13	6.101	1.337	(-------*------)
C. pettiti	44	8.470	2.555	(---*---)
D. modesta	8	7.291	3.239	(---------*--------)
H. alternans	18	6.055	2.448	(-----*------)
H. betteni	42	6.456	1.445	(---*---)
H. slossonae	40	7.620	2.199	(----*---)
H. sparna	31	7.057	2.340	(----*----)
P. apicalis	18	4.220	0.565	(-----*------)

4.5 6.0 7.5 9.0

5.3.3.3 Terrestrial fatty acid markers

Terrestrial fatty acid markers included: 18:3ω3 + 18:2ω6 > 2.5, 22:0 + 24:0, and ΣC24:0 to C32:0 (Appendix 5 (section 10.5)). Their use was limited in this study because proportions of 22:0 and 24:0 were low (~0.5%) and did not differ among species, so this sum was not used as a fatty acid marker. Using the ΣC24:0 to Ç32:0 was not appropriate as only 24:0 was quantified because longer chain fatty acids were not present in the samples. The fatty acid marker of 18:3ω3 + 18:2ω6 was used because proportions were greater than 2.5 (~15-20%) and differed significantly among species (Table 5.26). Considering species individually, only *A. ladogensis* showed a seasonal difference with higher proportions in summer ($p<0.0001$) than in spring. By location, *H. betteni* ($p=0.039$), *H. slossonae* ($p=0.011$) and *H. sparna* ($p<0.0001$ summer only) had higher proportions of the terrestrial fatty acid marker downstream than at outlets. There were no significant differences by landscape or life stage.

Table 5.26 One way ANOVAs for Terrestrial fatty acid markers

One-way ANOVA: Terrestrial (18:3ω3+18:2ω6) versus Species

Source	DF	SS	MS	F	P
Species	7	571.1	81.6	3.58	0.001
Error	206	4692.6	22.8		
Total	213	5263.7			

S = 4.773 R-Sq = 10.85% R-Sq(adj) = 7.82%

Individual 95% CIs For Mean Based on Pooled StDev of 4.773

Level	N	Mean	StDev	----+---------+---------+---------+-----
A. ladogensis	13	16.910	6.486	(----------*---------)
C. pettiti	44	15.651	4.558	(-----*----)
D. modesta	8	17.678	3.592	(-------------*------------)
H. alternans	18	17.271	5.688	(--------*--------)
H. betteni	42	15.518	3.241	(-----*-----)
H. slossonae	40	15.915	4.857	(-----*-----)
H. sparna	31	19.332	6.020	(-----*------)
P. apicalis	18	20.174	3.566	(--------*--------)

----+---------+---------+---------+-----

15.0 17.5 20.0 22.5

5.3.3.4 Carnivory fatty acid markers

There are several carnivory fatty acid markers including: 20:1+22:1, 18:1ω9, 18:1ω7/18:1ω9 <1, 18:1ω9/(18:1ω7 + 16:1ω7) >1, 20:5ω3/22:6ω3 <1, PUFA/SAFA >1 (Appendix 5 (section 10.5)). Application of these markers to the trophic level of freshwater hydropsychids was difficult because knowledge of these markers is derived from marine environments and at higher levels in the food chain. The sum of 20:1+22:1 was very low (~0.2%) and the ratio of 20:5ω3/22:6ω3 was very high (>30) and so these two markers were not used. The fatty acid 18:1ω9 is not a unique marker of carnivory and so was not used. Present in samples was 16:1ω7 so the ratio 18:1ω9/(18:1ω7 + 16:1ω7) was more applicable than 18:1ω9/18:1ω7. Also used was the PUFA/SAFA ratio as values were greater than one.

Both of these fatty acid markers differed significantly among species but did not follow a similar trend (Table 5.27). Considering the 18:1ω9/(18:1ω7 + 16:1ω7) ratio for each species, *H. betteni* (p=0.006), *H. sparna* (p=0.003) and *A. ladogensis* (p=0.002) had

higher proportions in the summer compared to the spring, and *H. sparna* (p=0.003) had higher proportions downstream compared to outlets with no interaction with season. Considering the PUFA/SAFA ratio with species, only *H. sparna* (p=0.005) and *A. ladogensis* (p<0.0001) had higher proportions in the summer compared to the spring, and *C. pettiti* had higher proportions in barren landscapes (p=0.007) than forested landscapes. Thus only *H. sparna* and *A. ladogensis* having higher proportions in the summer agree using both markers. There were no significant differences by location or life stage.

Table 5.27 One way ANOVAs for Carnivory fatty acid markers

One-way ANOVA: Carnivory 18:1ω9/(16:1ω7+18:1ω7) versus Species

```
Source     DF       SS      MS      F      P
Species     7   19.892   2.842   5.45  0.000
Error     206  107.491   0.522
Total     213  127.382
S = 0.7224   R-Sq = 15.62%   R-Sq(adj) = 12.75%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.7224
Level          N    Mean   StDev  ----+---------+---------+---------+-----
A. ladogensis  13  1.8341  0.9291               (-------*-------)
C. pettiti     44  2.0209  0.6487                     (---*----)
D. modesta      8  1.8111  0.8393            (---------*---------)
H. alternans   18  2.0421  0.7291                     (---*-------)
H. betteni     42  2.3627  0.9697                               (---*----)
H. slossonae   40  1.8575  0.5199                   (---*----)
H. sparna      31  1.9450  0.6639                    (----*----)
P. apicalis    18  1.1439  0.4024  (------*------)
                                  ----+---------+---------+---------+-----
                                    1.00      1.50      2.00      2.50
```

One-way ANOVA: P/S versus Species

```
Source     DF       SS      MS     F      P
Species     7   2.4280  0.3469  3.97  0.000
Error     206  17.9859  0.0873
Total     213  20.4138
S = 0.2955   R-Sq = 11.89%   R-Sq(adj) = 8.90%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.2955
Level          N    Mean   StDev  -------+---------+---------+---------+--
A. ladogensis  13  1.4405  0.3428             (-------*-------)
C. pettiti     44  1.2780  0.2277          (---*---)
D. modesta      8  1.2688  0.2094  (---------*----------)
H. alternans   18  1.6031  0.4318                       (------*------)
H. betteni     42  1.2848  0.3015          (---*----)
H. slossonae   40  1.3117  0.2586           (----*---)
H. sparna      31  1.4085  0.3480               (----*-----)
P. apicalis    18  1.5292  0.2302                    (-----*------)
                                  -------+---------+---------+---------+--
                                       1.20      1.40      1.60      1.80
```

5.3.3.5 Essential fatty acid markers

The fatty acid marker entitled "all essential" was a summation of all the essential fatty acids (18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3, 22:5ω3, 22:6ω3), whereas "essential HUFA" only summed the last four fatty acids. Both markers were significantly different among species, being highest in *H. alternans* and lowest in *D. modesta* (Table 5.28). Considering species separately, *A. ladogensis* had higher proportions ($p<0.0001$) of the "all essential" fatty acid marker in the summer, than in the spring, as did *H. sparna*, but there was a significant interaction with location where outlets had lower proportions ($p<0.0001$) than downstream locations. There were no general trends in terms of significant comparisons amongst species using the "essential HUFA" marker (Table 5.28). Considering species individually by season, only *A. ladogensis* ($p=0.037$) had higher proportions in the summer compared to the spring samples. By landscape, only *H. alternans* ($p=0.018$) had higher proportions in forested compared to barren landscapes. By location, only *H. betteni* ($p=0.007$) had higher proportions at outlets compared to downstream samples.

Diplectrona modesta generally had lower proportions of these fatty acids compared to the other species (Table 5.28). *Parapsyche apicalis* had higher proportions of 18:2ω6 and lower proportions of 18:3ω3 and 20:4ω6 and showed considerable variation compared to other species (Table 5.28). *Arctopsyche ladogensis* and *H. alternans* were generally at the higher end of the spectrum for many of the fatty acids in Table 5.28. Of the three species usually found at outlets, *C. pettiti* and *H. betteni* were consistently similar to each other in terms of their proportions of these fatty acids, and generally had lower proportions than *H. alternans* (Table 5.28). *Hydropsyche slossonae*

and *H. sparna* were also similar to each other in terms of their proportions of these fatty acids and showed less variation than the other species (Table 5.28).

Table 5.28 One way ANOVAs for Essential fatty acid markers

One-way ANOVA: all essential versus Species

```
Source    DF      SS     MS     F      P
Species    7   910.5  130.1  4.72  0.000
Error    206  5679.7   27.6
Total    213  6590.1
S = 5.251   R-Sq = 13.82%   R-Sq(adj) = 10.89%
                      Individual 95% CIs For Mean Based on Pooled StDev of 5.251
Level           N    Mean  StDev  ----+---------+---------+---------+-----
A. ladogensis  13  34.895  7.673                  (--------*--------)
C. pettiti     44  31.541  4.548             (---*----)
D. modesta      8  30.415  3.383  (----------*---------)
H. alternans   18  37.171  6.529                           (------*------)
H. betteni     42  32.369  4.950               (---*----)
H. slossonae   40  31.998  4.588              (---*----)
H. sparna      31  34.572  6.027                    (-----*----)
P. apicalis    18  37.033  4.627                           (------*------)
                                  ----+---------+---------+---------+-----
                                    28.0      31.5      35.0      38.5
```

One-way ANOVA: essential HUFA versus Species

```
Source    DF      SS    MS     F      P
Species    7   429.3  61.3  4.42  0.000
Error    206  2861.0  13.9
Total    213  3290.3
S = 3.727   R-Sq = 13.05%   R-Sq(adj) = 10.09%
                      Individual 95% CIs For Mean Based on Pooled StDev of 3.727
Level           N    Mean  StDev  ------+---------+---------+---------+---
A. ladogensis  13  17.986  2.495                       (------*------)
C. pettiti     44  15.891  3.508                   (---*---)
D. modesta      8  12.738  2.505  (-------*--------)
H. alternans   18  19.899  5.265                               (----*-----)
H. betteni     42  16.850  3.570                     (---*---)
H. slossonae   40  16.081  3.993                    (---*--)
H. sparna      31  15.240  4.098                 (----*---)
P. apicalis    18  16.858  2.351                    (-----*-----)
                                  ------+---------+---------+---------+---
                                    12.0      15.0      18.0      21.0
```

One-way ANOVA: 18:2ω6 versus Species

```
Source    DF      SS     MS      F      P
Species    7  647.79  92.54  23.69  0.000
Error    206  804.82   3.91
Total    213 1452.61
S = 1.977   R-Sq = 44.59%   R-Sq(adj) = 42.71%
                      Individual 95% CIs For Mean Based on Pooled StDev of 1.977
Level           N    Mean  StDev  -----+---------+---------+---------+-----
A. ladogensis  13   5.155  1.624   (----*---)
C. pettiti     44   4.938  1.473    (--*-)
D. modesta      8   6.515  2.719        (----*-----)
H. alternans   18   4.746  1.282  (---*---)
H. betteni     42   5.494  1.326       (-*-)
H. slossonae   40   5.352  2.008      (-*--)
H. sparna      31   5.398  1.776      (--*-)
P. apicalis    18  11.421  4.030                              (---*--)
                                  -----+---------+---------+---------+-----
                                     5.0       7.5      10.0      12.5
```

One-way ANOVA: 18:3ω3 versus Species

```
Source    DF      SS    MS     F      P
Species    7   456.7  65.2  4.01  0.000
Error    206  3348.2  16.3
Total    213  3804.9
S = 4.032   R-Sq = 12.00%   R-Sq(adj) = 9.01%
                    Individual 95% CIs For Mean Based on Pooled StDev of 4.032
Level          N    Mean  StDev  --+---------+---------+---------+-------
A. ladogensis  13  11.755  5.050            (--------*--------)
C. pettiti     44  10.713  4.121            (----*----)
D. modesta      8  11.163  3.628       (-----------*-----------)
H. alternans   18  12.527  4.837                 (------*-------)
H. betteni     42  10.024  3.073          (----*----)
H. slossonae   40  10.564  4.090            (----*----)
H. sparna      31  13.932  5.056                      (-----*----)
P. apicalis    18   8.753  0.955  (------*-------)
                                 --+---------+---------+---------+-------
                                 7.5      10.0      12.5      15.0
```

One-way ANOVA: 20:4ω6 versus Species

```
Source    DF      SS     MS     F      P
Species    7   72.15  10.31  4.89  0.000
Error    206  434.06   2.11
Total    213  506.21
S = 1.452   R-Sq = 14.25%   R-Sq(adj) = 11.34%
                    Individual 95% CIs For Mean Based on Pooled StDev of 1.452
Level          N   Mean  StDev  ---------+---------+---------+---------+
A. ladogensis  13  3.685  0.828                    (-------*-------)
C. pettiti     44  3.084  1.301                  (---*---)
D. modesta      8  2.106  1.020  (---------*---------)
H. alternans   18  3.848  1.926                       (-----*------)
H. betteni     42  3.948  1.867                         (---*----)
H. slossonae   40  3.047  1.148                 (---*----)
H. sparna      31  3.259  1.647                  (-----*----)
P. apicalis    18  1.970  0.646    (------*-----)
                                 ---------+---------+---------+---------+
                                      2.0       3.0       4.0       5.0
```

One-way ANOVA: 20:5ω3 versus Species

```
Source    DF      SS     MS     F      P
Species    7  292.49  41.78  5.41  0.000
Error    206 1592.25   7.73
Total    213 1884.74
S = 2.780   R-Sq = 15.52%   R-Sq(adj) = 12.65%
                    Individual 95% CIs For Mean Based on Pooled StDev of 2.780
Level          N    Mean  StDev  ---------+---------+---------+---------+
A. ladogensis  13  13.365  1.998                    (-------*------)
C. pettiti     44  12.088  2.660                  (---*----)
D. modesta      8  10.204  2.385  (---------*---------)
H. alternans   18  14.921  4.635                            (------*-----)
H. betteni     42  12.054  2.465                  (---*---)
H. slossonae   40  12.039  2.539                  (---*----)
H. sparna      31  11.392  2.917             (----*----)
P. apicalis    18  14.528  2.157                         (------*-----)
                                 ---------+---------+---------+---------+
                                       10.0      12.0      14.0      16.0
```

One-way ANOVA: 22:5ω3 versus Species

```
Source    DF       SS       MS     F      P
Species    7   1.2313   0.1759  2.02  0.054
Error    206  17.9026   0.0869
Total    213  19.1339
S = 0.2948   R-Sq = 6.44%   R-Sq(adj) = 3.26%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.2948
Level          N    Mean   StDev     +---------+---------+---------+---------
A. ladogensis  13  0.1600  0.2044               (---------*---------)
C. pettiti     44  0.0868  0.1083               (----*-----)
D. modesta      8  0.0438  0.0650     (------------*------------)
H. alternans   18  0.0861  0.0844             (-------*--------)
H. betteni     42  0.0831  0.1512               (----*-----)
H. slossonae   40  0.2175  0.5707                        (-----*----)
H. sparna      31  0.0794  0.1914              (------*-----)
P. apicalis    18  0.3072  0.3438                          (-------*--------)
                                  +---------+---------+---------+---------
                                -0.16      0.00      0.16      0.32
```

One-way ANOVA: 22:6ω3 versus Species

```
Source    DF       SS      MS     F      P
Species    7   11.840   1.691  3.98  0.000
Error    206   87.634   0.425
Total    213   99.473
S = 0.6522   R-Sq = 11.90%   R-Sq(adj) = 8.91%
                    Individual 95% CIs For Mean Based on Pooled StDev of 0.6522
Level          N    Mean   StDev  ------+---------+---------+---------+---
A. ladogensis  13  0.7754  0.4846                  (--------*--------)
C. pettiti     44  0.6318  0.5248                   (----*----)
D. modesta      8  0.3825  0.3702       (-----------*----------)
H. alternans   18  1.0439  0.8488                            (------*-------)
H. betteni     42  0.7660  0.7320                      (----*----)
H. slossonae   40  0.7760  0.7617                      (----*----)
H. sparna      31  0.5094  0.7106                 (-----*-----)
P. apicalis    18  0.0517  0.0406  (------*-------)
                                  ------+---------+---------+---------+---
                                      0.00      0.40      0.80      1.20
```

5.3.3.6 Summation of fatty acid marker results and comparison of co-occurring species

There were few clearly distinct patterns in terms of fatty acid markers with season, location, landscape and life stage (Table 5.29). Overall, some of the fatty acid markers for diatoms, dinoflagellates and green algae fatty acid markers tended to be higher in the spring than in the summer although this varied among hydropsychid species. Carnivory fatty acid markers showed a higher proportion in the summer than in the spring for three species (*H. betteni, H. sparna, A. ladogensis*). Overall, fatty acid markers

showed very few differences by location, although a few species showed higher levels of diatom markers. Fatty acid markers for HUFA were higher at outlets than at downstream sites for *H. betteni*. Only two species (*H. betteni* and *H. slossonae*) had higher proportions of terrestrial material in their diet downstream than at outlets. There were also few differences between forested and barren landscapes, with some species having higher proportions of general algae fatty acid markers, but this was not supported by having higher proportions of fatty acid markers for any of the algal groups. Differences between larvae and pupae were uncommon indicating that these species did not consistently metabolize fatty acids during metamorphoses.

Principal component analyses were used to compare fatty acid markers in co-occurring species at sites where sufficient samples were collected of multiple species: Watern outlet, Watern downstream and Barking Kettle outlet. These sites showed no clear distinction among species or life stage. A seasonal difference was evident at Barking Kettle outlet, where summer samples had lower amounts of the Leveille diatom and green algae (ω3/ω6) markers and higher amounts of the carnivory (18:1ω9/ (16:1ω7 + 18:1ω7)) and golden brown algae (16:0+18:1ω9) markers than spring samples. At the other two sites, Watern outlet or Watern downstream, no seasonal differences were evident.

Table 5.29 A summary of significant differences in fatty acid markers between factors (season, landscape, location, life stage) for individual species. Species name abbreviations are given below the table. Interactions among two or more factors are not included.

Fatty acid marker		Season		Landscape		Location		Life Stage	
		spring	summer	forested	barren	outlet	downstream	larvae	pupae
General algae	PUFA		arct	slss arct	chm				
	sum ω3		arct	slss arct					
Diatoms	20:5ω3					bett		para	
	16:4ω1					spr			
	$\Sigma C_{16}/\Sigma C_{18}$	arct dplc spr			spr	spr			alt arct
	Leveille diatoms	chm spr slss alt arct				slss			alt arct
Dinoflagellates	22:6ω3		alt						
	$\Sigma C_{18} + \Sigma C_{22}$ PUFA	chm spr slss arct				slss			
Green	18:3ω3		arct	arct			slss		chm
	ω3/ω6	bett spr alt							
Golden brown	16:0+18:1ω9			chm					
Cyanobacteria	18:1ω7		chm bett						
Bacteria	sum (see text)								
Terrestrial	18:3ω3+18:2ω6		arct				bett slss		
Carnivory	18:1ω9/(16:1ω7+18:1ω7)		bett spr arct				spr		
	PUFA/SAFA (P/S)		spr arct		chm				
all essential	sum (see text)		arct						
essential hufa	sum (see text)		arct	alt		bett			

where: alt = *H. alternans*, arct = *A. ladogensis*, bett = *H. betteni*, chm = *C. pettiti*, dplc = *D. modesta*, para = *P. apicalis*, slss = *H. slossonae*, spr = *H. sparna*

5.4 Discussion

5.4.1 Lipid Classes in Hydropsychidae

Four lipid classes accounted for most of the composition (94.0%) in hydropsychids, with TAG having the highest proportion (65.3%), then PL (16.0%), then free fatty acids (6.6%) and acetone mobile polar lipids (6.1%). The remaining lipid classes were either absent or had a very low percentage with only hydrocarbons and sterols having proportions above one percent. Lipid classes of hydropsychids consisted mostly of a neutral lipid (TAG), and polar lipids (AMPL & PL) (87.4%).

Tricacylglycerol is a storage lipid and is the largest proportion of lipid classes in most insects (Stanley-Samuelson et al. 1988). Since TAG is an energy reserve it is mobilized during periods of high demand such as starvation or reproduction, but here TAG reserves were high so hydropsychids appeared not to be nutritionally stressed. During periods of non-stress the fatty acid composition of TAG is similar to dietary intake (Dalsgaard et al. 2003). A higher level of TAG at outlets is a significant finding that indicates hydropsychids in these habitats have the ability to increase their lipid reserves because of higher quality and/or an excess of food. It was postulated that late fifth instar larvae would have elevated proportions of TAG because it may be utilized as an energy source for pupation and subsequent adult reproduction and dispersal. However, there were no significant differences in TAG between larvae and pupae. This suggests that the proportion of TAG in the total lipid classes may remain relatively constant over the later phases of larval and pupal development.

In the spring samples collected at outlets, the increase in the proportion of TAG was balanced by a decrease in the proportion of phospholipid (PL). A decrease in PL indicates the decreased need for growth because phospholipids are structural components important for somatic growth (Parrish et al. 2000). Therefore larvae collected in the late spring, when collected, larvae were likely full grown. In summer, the lack of a significant difference by location in PL concentration indicates that larvae were still undergoing development as reflected by the wide range of instars present at a site (Chapter 4). However, outlets have a high food supply and the greater proportion of TAG to PL in spring indicated that outlet larvae were further advanced in depositing storage lipids than larvae downstream.

Elevated levels of free fatty acids are indicative of sample decomposition. However, the mean free fatty acid amount in this study (6.6%) fell within the range of 5% to 25% in marine phytoplankton and zooplankton samples that were considered not to be decomposed (Parrish 1988). Three of the 65 samples had a fatty acid amount greater than 25% but there were no comparative values published for aquatic insects.

Polar lipid classes consist mostly of ω3 and ω6 PUFAs, which are important in the structure of cell membranes because they increase membrane fluidity at low temperatures (Dalsgaard et al. 2003). Thus higher levels of polar lipids are expected when analyzing the total lipid composition of the whole body of an organism because of their structural role. Time and resources permitting, it would have been beneficial to consider the fatty acid composition of TAG separately because TAG would have reflected hydropsychid dietary intake only, without the overlap of the PL structural

components. It is not clear if hydropsychids are able to synthesize long chain hydrocarbons, especially ω3 and ω6 PUFAs, so measuring their uptake solely from the TAG portion would reflect dietary uptake which could be compared to whole body concentrations. If little is being ingested then they must be able to produce these fatty acids *de novo*.

5.4.2 Fatty acid composition among and within species and life stages

There were several objectives in the fatty acid analysis of this study. One, could species be differentiated based on their fatty acid composition; two, did the larvae and pupae of a species differ in terms of their fatty acid composition; and three, could diet among species be differentiated using fatty acid markers and was there an influence of outlets and/or landscape?

Almost all of the 14 dominant fatty acids (14:0, 14:1A, 16:0, 16:1ω9, 16:1ω7, 17:1, 18:0, 18:1ω9, 18:1ω7, 18:2ω6, 18:3ω3, 18:4ω3, 20:4ω6, and 20:5ω3), except for 17:1 (bacterial origin), found in the hydropsychids are present in freshwater algae. Freshwater algae are extremely diverse (Sheath & Wehr 2003) and the seston shows great variability in proportions of fatty acids present depending on the dominance of algae groups (Napolitano 1999). The current study showed hydropsychids did have higher proportions of 18:0, 18:1ω7 and 20:5ω3 than those reported for most groups of freshwater algae (Napolitano 1999).

All insects are able to synthesize 14:0, 16:0, 18:0 and 18:1, and there is potential for conversion of C18 to C20 PUFAs (Stanley-Samuelson et al. 1988). Insects are also able to desaturate 16:0 and 18:0 to 16:1ω9 and 18:1ω9 respectively (Stanley-Samuelson

et al. 1988). Most animals can also synthesize 16:1ω7 and 18:1ω7 (Arts et al. 2001). Linoleic acid (18:2ω6) is synthesized from 18:0 and is further elongated to produce 20:4ω6 in some insects (Stanley-Samuelson 1993). However, α-linolenic acid (18:3ω3, which is a precursor to 20:5ω3) cannot be synthesized by insects and must be of dietary origin (Stanley-Samuelson 1993). Thus nine of the 14 most abundant fatty acids in hydropsychids were similar to those reported for insects and other animals, which would contribute to the lack of differentiation among species. If hydropsychids are able to synthesize most of their major fatty acids then only precursors need be obtained from food and the dominant fatty acids would be synthesized to meet their requirements. For example, hydropsychids had higher proportions of 20:5ω3 than reported for most groups of freshwater algae except diatoms (Napolitano 1999), and so they may be storing this essential fatty acid or were capable of synthesizing it as suggested by Bell et al. (1994). However, the rate and extent of such synthesis is not known but Brett & Muller-Navarra (1997) suggest that it is likely too low to support optimum growth rates and so 20:5ω3 must be obtained from dietary sources. Therefore a question that needs to be addressed is: can hydropsychids synthesize fatty acids at a rate and/or in required amounts for physiology, growth and reproduction or must some proportion be obtained from the diet? This is likely true for longer chain PUFAs because most organisms require greater quantities than they can synthesize (Arts et al. 2001). Many studies have focused on the transfer of lipids from the diet into tissues (compiled in (Dalsgaard et al. 2003));(Cripps & Atkinson 2000; Smith et al. 1997) and similar studies are needed for stream invertebrates so that trophic relationships can be better understood. Effects of

temperature, light and nutrient levels on fatty acid production in freshwaters also need to be assessed to form a basis for studying the natural dynamics of trophic transfer in populations.

Although there are common fatty acids among insects, insect fatty acid composition does differ with diet (Hanson et al. 1985) and so investigation of feeding differences among hydropsychids was possible. Fatty acid composition among hydropsychid species showed very few clear differences, possibly because of inter-species variability and/or similarities in feeding habits. Sekino et al. (1997) measured the fatty acid composition (C_{14} to C_{18} fatty acids) of individuals of three species of freshwater zooplankton and found the intraspecific differences greater than the interspecific differences and so were not able to distinguish feeding differences among the species. However, in that study the largest sample size was seven. Sample sizes in the current study (18 to 40 samples per species, except for *D. modesta*) were large enough to adequately determine the variability in fatty acid composition within a species. All species had similar dominant fatty acids, and standard deviations associated with each fatty acid were similar among all species which demonstrates that these closely related hydropsychid species did have a common fatty acid composition. Variability within a species was high for the fatty acids that constituted lower proportions. The 14 dominant fatty acids showed less variation with a similar variance found among all species.

Species separations using discriminate function analysis showed that *P. apicalis* was quite distinct, but the other seven species were quite similar to each other with *A. ladogensis, H. alternans* and *D. modesta* somewhat separated from the four commonly

occurring species (*C. pettiti, H. betteni, H.sparna* and *H. slossonae*). However, high misclassification rates and the lower quantified distance from the other species showed that their fatty acid composition was less distinct than that of *P. apicalis*. The four commonly occurring species were very similar, with *H. slossonae* having the least separation as shown by the low percentage correctly classified. *Hydropsyche slossonae* was found at the second highest number of sites, after *H. sparna*, and was found throughout streams (Chapter 4) indicating its ability to exploit a wide range of resources. This was supported by its general fatty acid composition which was not distinct from the other species.

Hanson et al. (1985) used discriminate function analysis to classify the fatty acid composition of 58 aquatic genera of insects into seven orders and had a ~76% success rate. They found Trichoptera filter-feeders to have a significantly different fatty acid composition from all other members of this order. Thus there is evidence that the fatty acid composition of the Trichoptera is related to feeding method which reflects the food sources utilized.

The first reported fatty acid composition of *Hydropsyche* was by Moretti et al. (1976). They found a similar fatty acid composition to that of the current study, but because of technical problems they did not report proportions of longer chain fatty acids (>20). Hanson et al. (1985) added data from Moretti et al. (1976) authors to their 1985 paper but they were broken down into neutral lipids and phospholipids and so cannot be compared with this study.

In the Hanson et al. (1985) analyses of the fatty acid composition of seven orders of aquatic insects, the results were expressed in the same units as the current study but they did not report the position of the first double bond in a fatty acid. Six fatty acids had an unknown identity with the first occurring between 14:0 and 14:1, a similar position to the unknown fatty acid detected in the current study. Hanson et al. (1985) found Trichoptera to have high proportions of 18:3 and low proportions 20:4 and 20:5 compared to the other 6 orders. They had two samples of larval *Hydropsyche* sp. from the lower reaches of a Utah river and a comparison of means reported for these two samples to the 123 larval *Hydropsyche* samples here, showed that Newfoundland *Hydropsyche* had lower proportions of 14:0 and higher proportions of 20:4 and PUFA (α=0.05). Hanson et al. (1985) also analyzed six samples of larval *Parapsyche* sp., with two from a different Utah river and four from two different Oregon rivers. Comparing means reported to the 13 larval samples collected here, the Newfoundland samples had lower proportions of 16:0, 18:1, SAFA and MUFA and higher proportions of 18:2, 20:5 and PUFA (α=0.05). The higher proportions of the long chain fatty acids in Newfoundland samples may be because of colder temperatures requiring increased membrane fluidity as discussed above.

Sushchik et al. (2003) studied Trichoptera (Limnephilid and Macronema) fatty acid composition sampled from the Yenisei River in Siberia and pooled all ages of all Trichopteran species. The two most abundant fatty acids identified were 16:0 and 18:1ω9, with small amounts of two unusual fatty acids, 14:2 and 14:3. Fatty acid proportions were expressed as mg per gram wet weight of their samples and so cannot be

directly compared with this study, although relative proportions of the most abundant fatty acids were similar.

In the current study, species fatty acid compositions did differ, with the fatty acid composition of *P. apicalis* being the most distinct from the other species according to PCA and discriminate function analyses. This may be partly attributable to it only being collected in the spring because some of the variation in the fatty acid composition of other species was caused by seasonal changes in diet. *Parapsyche apicalis* was only collected in two streams and was the only hydropsychid that occurred in these streams. It is not known if its distinction from the other species was caused by the resources available in these two streams. It is known from the broad survey (Chapter 2) that *P. apicalis* occurred with other species and direct comparison with co-occurring species at these sites would be informative.

Parapsyche apicalis separated from the other species because of higher proportions of 16:1ω7, 16:4ω1 and 18:2ω6 and lower proportions of 17:1 and 20:4ω6 (however proportions of 20:4ω6 were similar in *D. modesta*). Palmitoleic acid (16:1ω7) can be indicative of diatoms in marine systems (Auel et al. 2002) and 16:4ω1 is also a general (freshwater and marine) diatom marker (Parrish et al. 2000). It is possible that diatom species in the two streams where *P. apicalis* occurred differed from those in the eight steams sampled where the other species occurred because *P. apicalis* is known to inhabit colder streams (Chapter 4) which may have different diatom assemblages (Sheath & Wehr 2003). Proportions of 16:1ω7 depend on the physiological state of the algae. Senescent cells stop converting 16:1ω7 into longer chain fatty acids, causing proportions

to increase which may also account for higher proportions in *P. apicalis* (Leveille et al. 1997).

Linoleic acid (18:2ω6) is synthesized by plants and is a precursor to several long chain ω3 and ω6 PUFAs including arachidonic acid (20:4ω6) (Dalsgaard et al. 2003). Thus higher proportions of linoleic acid in *P. apicalis* may be caused by lack of conversion into longer-chain PUFAs, such as arachidonic acid, which would also account for the lower proportions of that compound.

The odd carbon numbered mono-unsaturated fatty acid, 17:1, is indicative of bacteria (Budge & Parrish 1998) which may be a food source or part of the gut flora. Thus lower proportions of this fatty acid could be because of differential food intake and/or because gut processes may differ slightly in *P. apicalis*.

Diplectrona modesta had higher proportions of 20:4ω3 (eicosatetraeonic acid) than other species. This is a precursor to 20:5ω3 (eicosapentaeonic acid (EPA)), which was present in lower proportions in this species. Thus, this may be caused by differences in conversions along the ω3 pathway. *Diplectrona modesta* also had lower proportions of 20:4ω6 without a concurrent increase evident in any of its precursors, but this could still be caused by differences in conversion rates and/or abilities in this species and may not be caused by dietary differences. *Diplectrona modesta* larvae were mainly collected 110 m downstream from the outlet in Barking Kettle, a small stream surrounded by boreal coniferous forest. Seston at this site had a high concentration of amorphous material which may be lacking 20:5ω3. Adult black flies (Simuliidae) emerging from this site

were significantly smaller than those in nearby streams (Colbo 1982), indicating that food quality at Barking Kettle may be poor for filter feeding insects.

Arctopsyche ladogensis had higher proportions of 18:1ω7 (*cis*-vaccenic acid) and lower proportions of 14:1 than other species. *Cis*-vaccenic acid is present in minor amounts in freshwater eukaryotic algae and cyanobacteria (Napolitano 1999) so this species may utilize greater quantities and/or different species of these organisms than other hydropsychids. However, 18:1ω7 is also formed from elongation of 16:1ω7 which is present in diatoms (Auel et al. 2002), so higher proportions could also be because of differing processing abilities in this species.

Arctopsyche ladogensis and *H. alternans* had lower proportions of 14:1 compared to other species. This fatty acid has been reported in marine red algae (Jayasankar & Kulandaivelu 1999) and in low proportions in lake phytoplankton (14:1ω5) (Bourdier & Amblard 1988) but has had very little discussion in the literature. These two species may consume smaller quantities of organisms containing 14:1 than other hydropsychids. This was the only fatty acid that segregated *H. alternans* from *A. ladogensis, C. pettiti* and all other *Hydropsyche,* indicating that they had a very similar fatty acid composition.

Hydropsyche sparna had higher proportions of 18:3ω3 than *C. pettiti, H. betteni* and *H. slossonae*. This fatty acid is present in green plants, both aquatic and terrestrial plants including stream periphyton (Napolitano 1999). *Hydropsyche sparna* was widely distributed across all stream habitats (Chapter 4) and was found at the greatest number of sites in this study. Therefore, this species could obtain this fatty acid from a wide range of sources throughout the eight streams sampled here (Chapter 4) and the green algal

component of periphyton is a possible source of 18:3ω3 (Sheath & Wehr 2003). *Hydropsyche sparna* was found in higher abundances downstream than at outlets (Chapter 4), so it may be also consuming material derived from plants which would include 18:3ω3 from terrestrial plants. *Hydropsyche sparna* also had lower proportions of 18:1ω9 than *C. pettiti, H. betteni* and *H. slossonae*, a fatty acid which is common to bacteria, autotrophs and heterotrophs but in this study it was used as a carnivory marker because it was found in higher proportions than 18:1ω7 (Falk-Petersen et al. 2000). Thus *H. sparna* may consume less animal material than other species. This was in contrast to *H. betteni*, which had a higher proportion of 18:1ω9, and is reported to be more carnivorous than other *Hydropsyche* (Fuller & MacKay 1980a). These results support this difference in food resource use.

The four most commonly occurring species (*C. pettiti, H. betteni, H. sparna* and *H. slossonae*) generally had very similar fatty acid compositions. This may be an artifact of their occurrence in multiple streams where they may have utilized many food sources which would have caused their fatty acid compositions to be non-distinct. This would cause fatty acid proportions to be highly variable within a species which would obscure differences among species. Several species at a site had similar fatty acid compositions suggesting that they had a similar diet. This was observed for *C. pettiti* and *H. betteni* which commonly occurred together at outlets and showed no distinction from each other, indicating little food partitioning. Similar results were seen in a comparison of *H. sparna* and *H. slossonae* which commonly occurred together at downstream locations, but showed only slight differences in their fatty acid composition, suggesting similar dietary

sources. Furthermore, there was not a clear distinction between outlet (*C. pettiti* and *H. betteni*) and downstream species (*H. sparna* and *H. slossonae*) indicating that food did not greatly differ between outlets and downstream locations. There was also not a clear distinction between forested and barren landscapes. Differences in diet, determined using fatty acids, in studies on other aquatic organisms were associated with more distinct niche separation, such as occurring at greater depths or in open water versus shoreline habitats (Auel et al. 2002; Jayasankar & Kulandaivelu 1999; Scott et al. 1999).

Zooplankton abundances were higher near outlets (Chapter 4), but fatty acid markers of carnivory were not found to be elevated at outlets. Thus hydropsychids appear to ingest equivalent quantities of animal material regardless of its abundance in the seston. It is also possible that for freshwater habitats the carnivory fatty acid markers used were not specific indicators because they were developed in marine systems (Cripps & Atkinson 2000; Dalsgaard et al. 2003; Falk-Petersen et al. 2000; Stevens et al. 2004b). For example, in marine animals, 16:1ω7 and 18:1ω7 indicate dietary phytoplankton and 18:1ω9 indicates animal input and so the ratio of these fatty acids can be used as a carnivory marker (Falk-Petersen et al. 2000). In freshwater phytoplankton, only cyanobacteria contain both 16:1ω7 and 18:1ω7, and green algae and dinoflagellates have 18:1ω9 and thus this ratio may be less indicative of carnivory in freshwaters (Napolitano 1999). Antarctic krill fed copepods were found to increase their PUFA to SAFA ratio compared to those fed diatoms, and so ratios greater than one were considered evidence of a carnivorous diet (Cripps & Atkinson 2000). However, PUFA also aid in membrane fluidity in cold environments because they have low melting points compared to other

fatty acids, and so most poikilotherms increase their PUFA to SAFA ratio in response to colder ambient temperatures (Brett & Muller-Navarra 1997; Fast 1970). Here, *P. apicalis* inhabits colder streams (Chapter 4) and was found to have a higher ratio of PUFA to SAFA than the other hydropsychids. Thus this ratio would be a poor indicator of carnivory in these streams if it is greatly affected by temperature. The lack of an adequate carnivory marker means that dietary relationships cannot be accurately assessed in the current study. It has been noted that the use of fatty acid markers is more limited and complex when identifying food sources of carnivores and omnivores as opposed to herbivores (Dalsgaard et al. 2003). Hydropsychids are omnivores and thus feed across lower and higher trophic levels, weakening the clarity of the fatty acid 'signature' compared to zooplankton which were used to develop carnivory markers (Auel et al. 2002). This demonstrates the need for basic research on the lipid biochemistry of freshwater organisms to clarify the specificity of fatty acid markers and to develop better protocols for evaluation of analytical results.

Only three fatty acids (17:1, 18:2ω6, 20:4ω6) were consistently different between larvae and pupae for all species combined. These were also the fatty acids that separated *P. apicalis* from the other species when stage was not a factor. Thus *P. apicalis* separates using these fatty acids regardless of stage. No consistent trends were evident when analyzing larvae and pupae of individual species, indicating that fatty acid composition was not consistently altered upon pupal formation. Hanson et al. (1985) suggested that since long chain fatty acids (20:4ω6 and 20:5ω3) affect membrane fluidity and are essential for proper membrane function at low temperatures, later stages of aquatic

insects may decrease proportions of these fatty acids as they prepare to leave the colder aquatic environment. In this study, only *P. apicalis* pupae had lower proportions of 20:5ω3 than its larvae. *Cheumatopsyche pettiti* pupae did have higher amounts of 18:3ω3 but only slightly lower levels of 20:5ω3 which were not statistically different from levels in larvae. Newfoundland air temperatures are often very close to water temperatures, except in the summer for *P. apicalis* which inhabits colder streams (Flint 1961) (Chapter 4).

Separation by season was evident for *H. sparna* (Figure 5.8), *H. slossonae* (Figure 5.9) and *A. ladogensis* (Figure 5.12). For these three species the proportion of 14:0 and 18:4ω3 was higher in the spring and the proportion of 16:1ω9 and 20:4ω6 was higher in the summer suggesting a dietary shift that may reflect food availability or palatability. A similar trend may be present in *D. modesta* but more sampling over seasons is required.

The current study was able to gauge the potential variability in fatty acid composition because species were sampled in late spring and late summer, at multiple sites, and included larvae and pupae. The spring samples were fifth instars collected in late June which represented the previous summer's cohort that over wintered, and thus their fatty acid composition potentially reflected dietary intake over several months including winter and early spring. Hydropsychids in summer samples, collected in August, had developed from the current year's oviposition and thus their nutrient intake was from summer food sources only. This range of factors, coupled with limitations on the number of samples one could process, meant that definitive statistical testing of relationships between all factors could not be conducted.

Hydropsychids were sampled at lake outlets and downstream in eight streams of different sizes and surrounding vegetation patterns, thus a full range of potential food resources should have been available. Therefore if hydropsychids are opportunistic feeders, then their fatty acid composition should show considerable variation. Hydropsychid fatty acid composition was highly variable without clear, consistent differences both between streams and at a given location within a stream. This indicates that hydropsychids were highly omnivorous and could utilize available food resources which may have had a patchy distribution. If a species only ingested a limited range of foods then its fatty acid composition would remain similar among all streams. Alstad (1987) states that hydropsychid diets are generalized and broad and that very subtle interspecific differences may be important determinants of community composition. Here, hydropsychids may not have high inter-specific competition at a site if all species were able to utilize a range of food resources. Dietary specialization is a possible mechanism for patterns of occurrence, abundance and community composition, but there is little known about the degree of specialization needed to determine the coexistence or displacement of species (Alstad 1987). Cummins (1973) states that most aquatic insects are generalists, with their diet mostly depending on food availability, which provides little support for the food partitioning hypothesis unless it is only evident under high competitive pressure. The differences in fatty acid composition amongst the hydropsychids here were subtle, which emphasizes the similarity in diet among these species. This suggests that Newfoundland hydropsychids are opportunistic generalists.

5.5 Conclusion

Lipid class composition of Newfoundland Hydropsychidae was similar to that of other freshwater macroinvertebrates, consisting mainly of the storage lipid TAG. Their fatty acid composition was also similar to that found in other aquatic insects (Hanson et al. (1985), with a dominance of 14:0, 16:0, 16:1ω7, 17:1, 18:0, 18:1ω7, 18:2ω6, 18:3ω3, 18:4ω3, 20:4ω6 and 20:5ω3. It was possible to discriminate the species based on their fatty acid composition and thus interspecific differences were greater than those within a species. *Parapsyche apicalis* was the most distinctive from the other species in terms of its fatty acid composition, followed by *A. ladogensis, D. modesta* and *H. alternans.* Discrimination of the remaining four species was more difficult indicating the similarity in their lipid composition. These four were also the most commonly occurring and most abundant of the Newfoundland Hydropsychidae. Their broad distribution may be aided by their ability to be dietary generalists, exploiting the available food resources of any given stream. Based on our current knowledge, subtle interspecific differences indicated that Newfoundland hydropsychids were opportunistic generalists in terms of their dietary intake. However, much more research is required on uptake, biosynthesis, metabolism and storage of lipids and fatty acids by freshwater organisms, especially those at intermediate trophic levels, to strengthen lipid analysis as a tool in tracking trophic relationships in lotic environments. Some suggested first steps would be controlled feeding of specific diets to larvae in the laboratory, and the repeated sampling of one location over seasons analyzing both the hydropsychid taxa and the potential food sources.

6. CHAPTER 6: LIPID AND FATTY ACID COMPOSITION OF SESTON FROM EIGHT STREAMS ON THE AVALON PENINSULA OF NEWFOUNDLAND

6.1 Introduction

Seston is defined as organic matter, either living or non-living, suspended in the water column which is therefore a potential food source for filter-feeding organisms like Hydropsychidae. Lake outlets are rich in seston because of the outflow of lake phytoplankton and zooplankton (Chapter 4). With increasing distance downstream these materials decline and allochthonous inputs increase. These are primarily of terrestrial origin and thus the surrounding vegetation may influence the quantity and/or quality of the seston. The first objective of this chapter was to determine if seston composition differed from outlet to downstream and from forested to barren landscapes (section 1.10). The second objective was to evaluate the quantity and quality of available seston as a food source for hydropsychids.

Lipid and fatty acid composition analyses are a method of analyzing stream seston (Appendix 3 (section 10.3)), Appendix 5 (section 10.5), Chapter 5). Proportions of lipid classes are a measure of the proportions of structural, storage, decay and pigment lipids per seston sample. Fatty acid composition can be used to compare seston within stream locations and across landscapes. Fatty acid markers provide insight into the plankton composition (Appendix 5 (section 10.5)). Lipid analyses, combined with measures of the mass of organic matter at a site will be the tools used here to compare the seston of eight Newfoundland streams.

6.2 Materials and Methods

Seston was sampled in eight streams, at the outlet and downstream, in the late spring (June) and late summer (end of August) in 2004. The eight streams were located on the Avalon Peninsula of Newfoundland, with four in forested landscapes and four in barren sites (see Figure 4.1 for locations; Table 5.1 for site characteristics and Table 1.5 for descriptions of landscape types; Appendix 2 (section 10.2) for pictures of study sites).

Little is known of the lipid and fatty acid composition of freshwater seston and thus sampling in June was exploratory. At each site, 10 L of water was collected, avoiding bottom sediment and any large surface particles or films, in brown glass bottles pre-rinsed with stream water. The bottles were stored in a dark cooler on ice and transported to the lab. The water sample was divided in the lab into three 3 L samples and from the remaining water, three 250 mL samples were taken for seston weights.

Analyses of the spring samples revealed that they were not large enough, so in August 50 L samples were taken which necessitated filtration in the field. Water was sampled again, avoiding bottom sediments, surface films and large particles. The water collected was poured through a 10 μm mesh sieve in the field. The resulting material was washed into glass jars, pre-rinsed with double filtered water, and stored in a dark cooler on ice for transport back to the lab. Three 50 L samples were collected and filtered at each site for lipid analyses. Three 25 L samples were also collected and filtered at each site for seston weights.

In the lab, samples were filtered through Whatman GF/C glass fibre filters with a pore size of 1.2 μm. Filters with a diameter of 42.5 mm were used for the water samples for lipid analysis. These filters were previously burned in a muffle furnace at 400°F for

three to four hours to destroy any organic matter. Filters with a diameter of 23 mm were used for weights. Filters for the weights were previously dried for at least 24 hours at 27°C, weighed to the nearest microgram, and stored in labeled Petri dishes.

Water samples were filtered through Whatman GF/C filters by creating a vacuum using flowing water. A vacuum pump could not be used because of possible sample contamination by hydrocarbons. The resulting filtered water was refiltered to constitute a control for each sample undergoing lipid analyses. All equipment was rinsed with double filtered water before and after sample filtration both to avoid contamination and to ensure all material was retrieved.

For spring samples, 1 L (of the total 3 L) of water was sieved through one filter, and thus there were 3 filters per sample. Each sample had a corresponding 3 L control, filtered onto one filter. For summer samples the material was filtered onto as few filters as possible, and did not exceed three. One control was taken per site, the volume of which varied depending on the amount of material collected from the site.

Each filter was folded using forceps to avoid contamination and placed into a labeled test tube, that was pre-rinsed three times with methanol and three times with chloroform to remove any lipid contaminants. Two mL of chloroform was added to each test tube which was then capped under nitrogen, sealed with Teflon tape and stored at -20°C until extraction.

Lipids were extracted using the same method as for the Hydropsychidae samples (see section 5.2.2 for details). Seston samples had lower lipid proportions than hydropsychid samples and thus extract volumes were concentrated to 0.5 mL instead of

1.5mL prior to iatroscan analysis. Samples and their corresponding controls were iatroscanned and then the value of the control was subtracted from the sample. The average of the three control replicates was used for the spring samples. Use of controls were necessary as the chemicals used in the extraction procedure can modify the lipid class profile of a sample because of the inherent presence of hydrocarbons in the chloroform used for sample extraction.

For the seston weights, as much water as possible was passed through the filter. Volumes filtered and remaining were recorded so measured weight could be extrapolated to the entire sample. Samples were kept frozen at -20°C until all samples were collected. Then the filters were transferred to labeled aluminum weigh-boats and dried at 80°C for at least 48 hours. Filters were weighed to the nearest microgram and the weight of the filter paper recorded previously was subtracted. This was done in triplicate at each site and the average was taken as the quantity per litre of seston at a site.

After samples were iatroscanned, they were derivatized into their component fatty acid methyl esters (FAME) and their fatty acid composition was determined (see section 5.2.2 for details).

6.3 Results

6.3.1 Seston Quantity

Ash free dry weights (AFDW) (i.e. the organic portion of the seston) did not differ by location ($p=0.611$, Figure 6.1) or landscape ($p=0.810$, Figure 6.2) when all streams were combined. There were significant differences among streams ($p<0.0001$, Figure 6.3), with Broad Cove having lower amounts of organic material than the other

streams. There were also significant differences by location within a stream. Beaver Pond ($p<0.0001$) and Split Rock ($p=0.0160$) had greater amounts of organic material at the outlet versus the downstream site, whereas the opposite was true for Great Pond ($p=0.0005$), Broad Cove ($p=0.0007$) and Portugal Cove ($p<0.0001$) which had more organic matter at the downstream site than at the outlet (Figure 6.3).

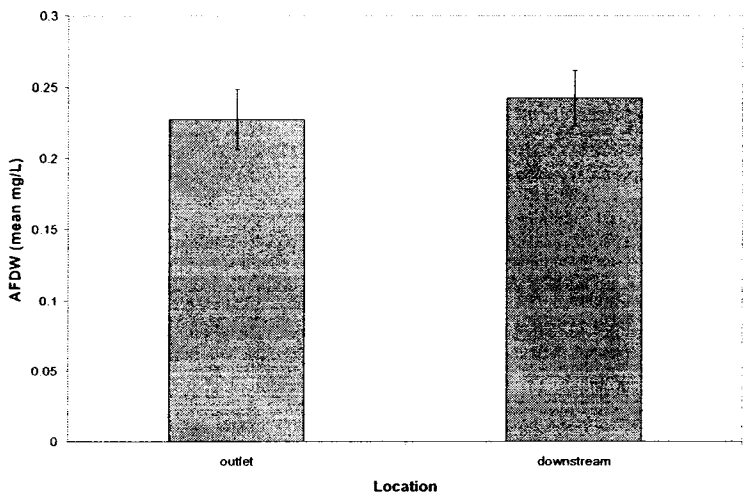

Figure 6.1 AFDW of seston by location for all streams combined in summer.

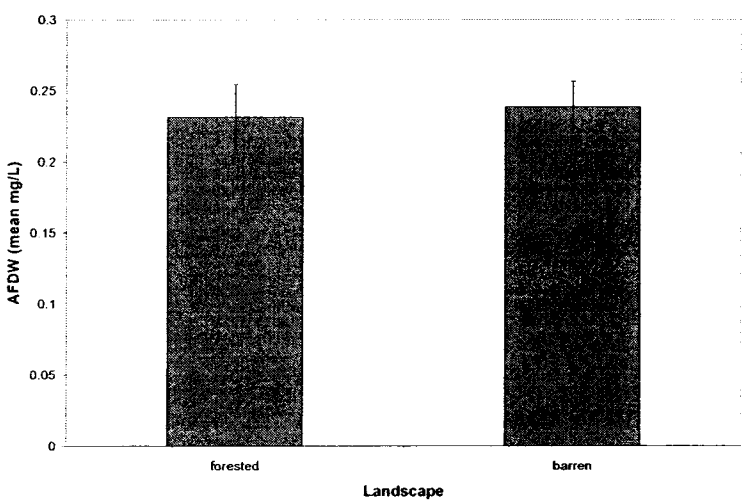

Figure 6.2 AFDW of seston by landscape for all streams combined in summer.

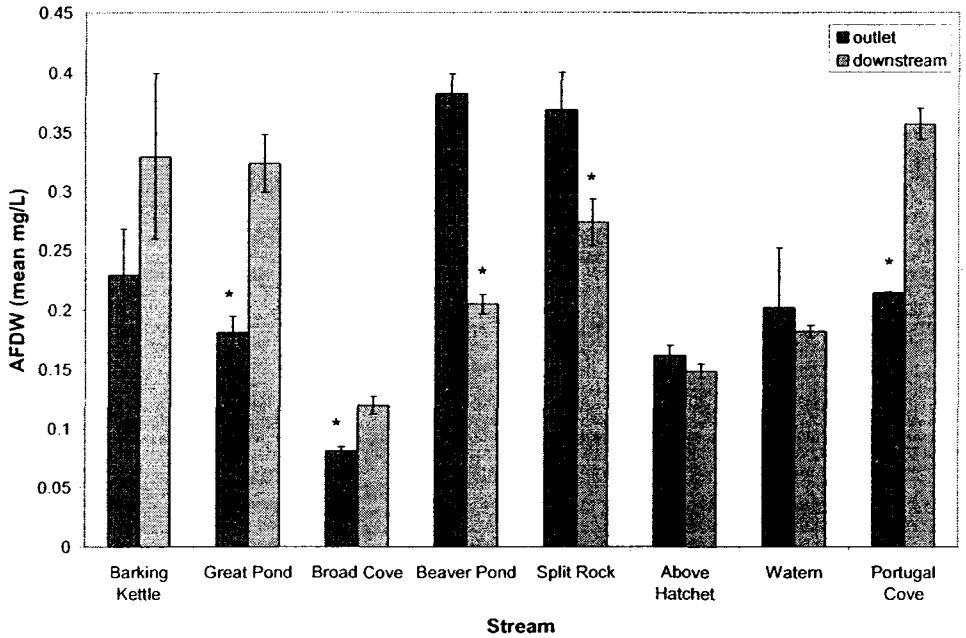

Figure 6.3 AFDW of seston by location within a stream in summer.

6.3.2 Seston Lipid Classes

As the spring and summer samples differed volumetrically, lipid classes were converted to a percentage of the total. This also allowed comparison with the Hydropsychidae lipid class composition (Chapter 7). Lipid classes of the seston differed between spring and summer (Figure 6.4). This data set combined all eight streams at outlets and downstream sites. The largest differences were between TAG and AMPL.

Further analysis of lipid classes showed no significant differences between outlet and downstream sites when all eight streams were combined. Hydrocarbons and fatty acids were significantly higher in forested landscapes, and ethyl ketones and AMPL were slightly but significantly higher in barren landscapes. Lipid classes differed among streams, but this significantly interacted with season. Therefore, separate analyses were required for each stream in each season which would not have added to the understanding

of these systems and so were not conducted. These preliminary data indicated seasonal differences in lipid composition of these freshwater streams, but since there were only two sampling times such indications must be viewed with caution.

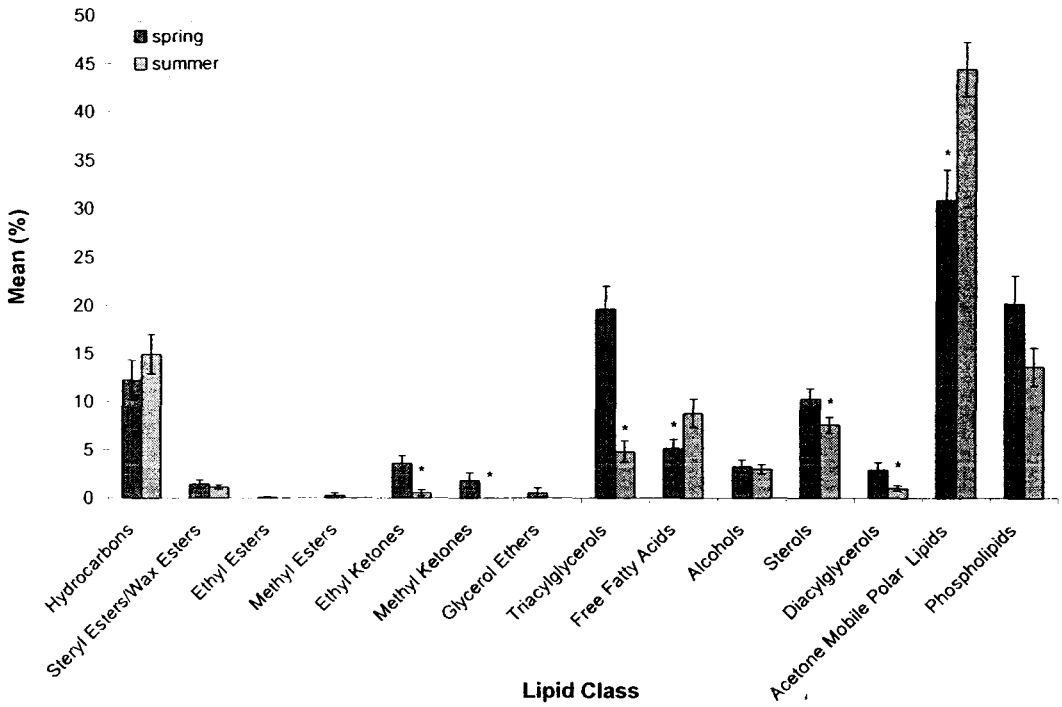

Figure 6.4 The percent lipid classes, a mean of 3 replicates, by season for all eight streams sampled. Asterisks denote significant differences.

Table 6.1 Mean and standard deviation (+/-) of each lipid class by season and overall.

	spring		summer		both seasons	
Lipid Class	mean	+/-	mean	+/-	mean	+/-
Hydrocarbons	12.2	14.2	14.9	14.2	13.6	14.2
Steryl Esters/Wax Esters	1.44	3.0	1.15	1.7	1.29	2.4
Ethyl Esters	0.12	0.6	0	0	0.06	0.4
Methyl Esters	0.28	1.7	0	0	0.14	1.2
Ethyl Ketones	3.61	5.4	0.55	2.1	2.07	4.4
Methyl Ketones	1.83	5.4	0	0	0.90	3.9
Glycerol Ethers	0.50	3.4	0	0	0.25	2.4
Triacylglycerols	19.6	16.6	4.84	7.5	12.2	14.8
Free Fatty Acids	5.16	6.2	8.81	10.0	7.01	8.5
Alcohols	3.30	4.5	3.00	3.3	3.15	3.9
Sterols	10.3	7.3	7.60	5.7	8.93	6.6
Diacylglycerols	2.94	5.0	1.03	2.0	1.98	3.9
Acetone Mobile Polar Lipids	30.9	21.6	44.5	19.4	37.8	21.5
Phospholipids	20.2	19.8	13.6	13.9	16.9	17.3

A principal component analysis of the lipid classes, including both seasons, showed the first three components only explained ~39% of the variation. Coefficients of the first and second components were low (~ < 0.5) indicating a low degree of differentiation among the lipid classes, with most samples having low proportions of methyl esters, alcohols, ethyl ketones and diacylglycerols (Figure 6.5). There was not a clear distinction between seasons, emphasizing their similar lipid class composition. However, summer samples showed less variability, possibly because of the larger volume of water filtered (Figure 6.6). There were no clear separations by location, landscape or stream which again indicated a similar lipid class composition regardless of these factors (Figure 6.6).

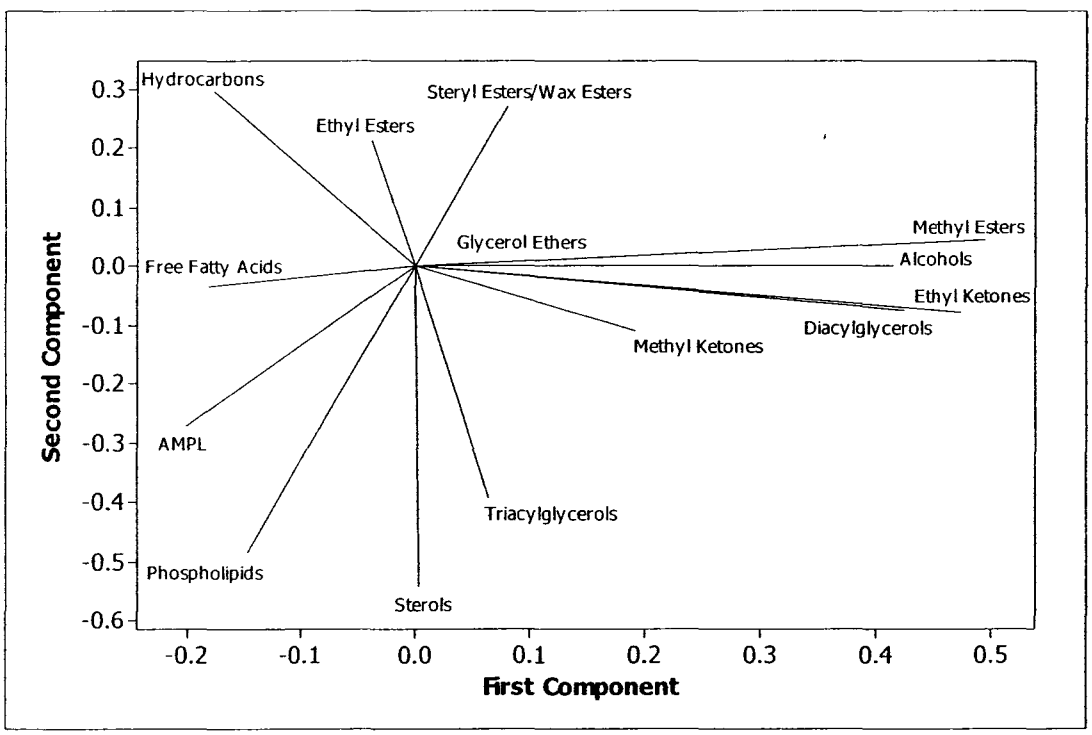

Figure 6.5 Loading plot of lipid classes using both seasons.

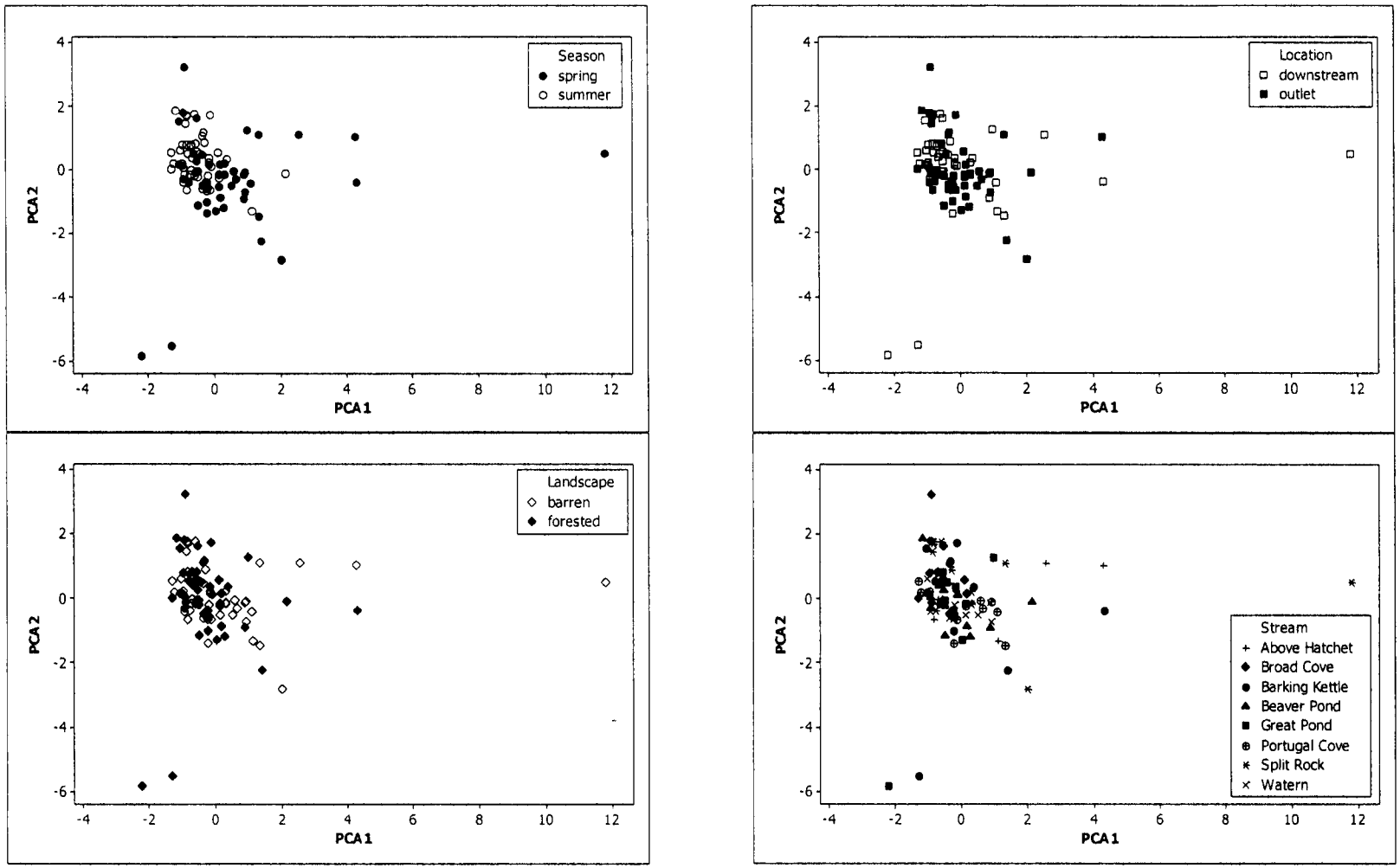

Figure 6.6 Score plots for seston samples by season, location, landscape and stream.

One-way ANOVAs showed no significant differences by location, but there were significant differences by landscape and by stream. However, these factors also had significant interactions with season. Considering each season separately for streams, lipid classes which differed significantly among streams in spring were: ethyl esters ($p=0.044$), ethyl ketones ($p=0.010$), TAG ($p<0.0001$) and free fatty acids ($p=0.030$) (Table 6.2). Great Pond had a much higher level of TAG compared to other streams (Table 6.2). Broad Cove and Great Pond had higher levels of free fatty acids compared to other streams (Table 6.2). In the summer, lipid classes which significantly differed among streams were: hydrocarbons ($p<0.0001$), steryl/wax esters ($p<0.0001$), free fatty acids ($p<0.0001$), AMPL ($p<0.0001$) and phospholipids ($p=0.005$) (Table 6.2). Hydrocarbons were elevated in Split Rock; free fatty acids were higher in forested streams (Broad Cove, Barking Kettle, Great Pond and Beaver Pond); AMPL was higher in Above Hatchet and Watern; and phospholipids were higher in Broad Cove and Portugal Cove (Table 6.2). These results demonstrate the difference among streams within a sampling season. There were no consistent patterns among streams or within a stream.

Table 6.2 One-way ANOVAs of lipid classes constituting at least 5% of the total among streams by season.

One-way ANOVA: Hydrocarbons versus Stream, spring

```
Source  DF    SS   MS     F      P
Stream   7  2269  324  1.79  0.116
Error   39  7047  181
Total   46  9317
S = 13.44   R-Sq = 24.36%   R-Sq(adj) = 10.78%
                  Individual 95% CIs For Mean Based on Pooled StDev of 13.44
Level          N   Mean  StDev  ------+---------+---------+---------+---
Above Hatchet  6  10.10  13.41       (---------*---------)
Barking Kettle 6  27.05  21.38                  (---------*---------)
Beaver Pond    6   8.08   9.77     (---------*---------)
Broad Cove     6  18.33  21.81               (---------*---------)
Great Pond     6  12.44  11.27          (---------*---------)
Portugal Cove  6   6.93   6.13     (---------*---------)
Split Rock     6   3.87   3.05   (---------*---------)
Watern         5  10.78   5.90       (---------*---------)
                                  ------+---------+---------+---------+---
                                        0        12        24        36
```

One-way ANOVA: Hydrocarbons versus Stream, summer

```
Source  DF      SS     MS      F      P
Stream   7  6377.4  911.1  11.77  0.000
Error   40  3097.1   77.4
Total   47  9474.5
S = 8.799   R-Sq = 67.31%   R-Sq(adj) = 61.59%
                    Individual 95% CIs For Mean Based on Pooled StDev of 8.799
Level           N    Mean   StDev  ----+---------+---------+---------+-----
Above Hatchet   6   0.690   1.040  (---*----)
Barking Kettle  6   9.879   7.660       (----*---)
Beaver Pond     6  21.588  14.727                (---*----)
Broad Cove      6  17.064   7.101            (---*----)
Great Pond      6  17.235   6.095            (---*----)
Portugal Cove   6   4.067   1.064   (----*----)
Split Rock      6  39.963  15.744                          (----*---)
Watern          6   8.952   2.496      (----*----)
                                    ----+---------+---------+---------+-----
                                        0         15        30        45
```

One-way ANOVA: Triacylglycerols versus Stream, spring

```
Source  DF     SS   MS     F      P
Stream   7   6300  900  5.52  0.000
Error   39   6354  163
Total   46  12654
S = 12.76   R-Sq = 49.79%   R-Sq(adj) = 40.77%
                    Individual 95% CIs For Mean Based on Pooled StDev of 12.76
Level           N   Mean  StDev  -+---------+---------+---------+--------
Above Hatchet   6  11.66   5.46    (------*------)
Barking Kettle  6   9.43  20.10  (------*------)
Beaver Pond     6  12.90   3.47    (------*------)
Broad Cove      6  26.77   8.69            (------*------)
Great Pond      6  46.56  18.37                    (------*------)
Portugal Cove   6  20.79  18.30         (------*------)
Split Rock      6  13.52   7.23    (------*------)
Watern          5  14.45   5.56    (-------*------)
                                -+---------+---------+---------+--------
                                 0         15        30        45
```

One-way ANOVA: Triacylglycerols versus Stream, summer

```
Source  DF      SS    MS     F      P
Stream   7   504.7  72.1  1.34  0.258
Error   40  2154.7  53.9
Total   47  2659.4
S = 7.340   R-Sq = 18.98%   R-Sq(adj) = 4.80%
                    Individual 95% CIs For Mean Based on Pooled StDev of 7.340
Level           N   Mean   StDev     +---------+---------+---------+---------
Above Hatchet   6  6.804  10.545               (---------*---------)
Barking Kettle  6  0.000   0.000     (---------*---------)
Beaver Pond     6  2.782   5.307          (---------*---------)
Broad Cove      6  4.334   5.120            (---------*---------)
Great Pond      6  9.574  11.820                     (---------*---------)
Portugal Cove   6  9.522   9.746                     (---------*---------)
Split Rock      6  2.673   3.884          (---------*---------)
Watern          6  3.021   3.950           (---------*---------)
                                +---------+---------+---------+---------
                               -6.0       0.0       6.0      12.0
```

One-way ANOVA: Free Fatty Acids versus Stream, spring

```
Source  DF      SS    MS     F      P
Stream   7   545.4  77.9  2.54  0.030
Error   39  1197.0  30.7
Total   46  1742.5
S = 5.540   R-Sq = 31.30%   R-Sq(adj) = 18.97%
                 Individual 95% CIs For Mean Based on Pooled StDev of 5.540
Level           N    Mean   StDev  -----+---------+---------+---------+----
Above Hatchet   6   3.355   4.065     (--------*--------)
Barking Kettle  6   2.069   5.068  (--------*--------)
Beaver Pond     6   2.219   3.197  (--------*---------)
Broad Cove      6  10.015   4.540                  (--------*--------)
Great Pond      6  11.523  11.211                     (--------*--------)
Portugal Cove   6   3.228   2.302    (--------*---------)
Split Rock      6   3.893   5.337      (--------*--------)
Watern          5   4.932   2.938       (---------*---------)
                                    -----+---------+---------+---------+----
                                       0.0       5.0      10.0      15.0
```

One-way ANOVA: Free Fatty Acids versus Stream, summer

```
Source  DF      SS     MS     F      P
Stream   7  2463.6  351.9  6.35  0.000
Error   40  2215.4   55.4
Total   47  4679.0
S = 7.442   R-Sq = 52.65%   R-Sq(adj) = 44.37%
                   Individual 95% CIs For Mean Based on Pooled StDev of 7.442
Level           N    Mean   StDev  --------+---------+---------+---------+-
Above Hatchet   6   0.000   0.000  (-------*-------)
Barking Kettle  6  18.504   2.855                          (-------*-------)
Beaver Pond     6  18.332   8.611                          (-------*-------)
Broad Cove      6  14.481  11.525                      (-------*-------)
Great Pond      6   3.934   6.209       (-------*-------)
Portugal Cove   6  10.889  12.763               (-------*-------)
Split Rock      6   2.569   4.197      (-------*-------)
Watern          6   1.800   2.983     (-------*-------)
                                    --------+---------+---------+---------+-
                                          0.0       8.0      16.0      24.0
```

One-way ANOVA: Acetone Mobile Polar Lipids versus Stream, spring

```
Source  DF      SS   MS     F      P
Stream   7    1028  147  0.28  0.958
Error   39   20421  524
Total   46   21449
S = 22.88   R-Sq = 4.79%   R-Sq(adj) = 0.00%
                  Individual 95% CIs For Mean Based on Pooled StDev of 22.88
Level           N   Mean  StDev  ---------+---------+---------+---------+
Above Hatchet   6  28.74   8.15        (-----------*-----------)
Barking Kettle  6  34.05  44.85           (-----------*-----------)
Beaver Pond     6  35.37   7.39            (-----------*-----------)
Broad Cove      6  27.53  11.18       (-----------*-----------)
Great Pond      6  28.96  32.35        (-----------*-----------)
Portugal Cove   6  29.61  10.86        (-----------*-----------)
Split Rock      6  38.92  25.63              (-----------*-----------)
Watern          5  22.73   2.55  (------------*------------)
                                 ---------+---------+---------+---------+
                                         15        30        45        60
```

One-way ANOVA: Acetone Mobile Polar Lipids versus Stream, summer

```
Source  DF     SS    MS      F      P
Stream   7  12334  1762  13.29  0.000
Error   40   5304   133
Total   47  17637
S = 11.51   R-Sq = 69.93%   R-Sq(adj) = 64.67%
                      Individual 95% CIs For Mean Based on Pooled StDev of 11.51
Level           N   Mean  StDev  ---+---------+---------+---------+------
Above Hatchet   6  71.96  18.13                          (----*----)
Barking Kettle  6  45.13  11.70              (----*---)
Beaver Pond     6  23.92   8.61  (----*----)
Broad Cove      6  25.90   3.72   (----*----)
Great Pond      6  40.93   9.16           (---*----)
Portugal Cove   6  42.92  10.74            (---*----)
Split Rock      6  38.51   9.44          (---*----)
Watern          6  66.42  14.80                      (----*----)
                                    ---+---------+---------+---------+------
                                      20        40        60        80
```

One-way ANOVA: Phospholipids versus Stream, spring

```
Source  DF     SS   MS     F      P
Stream   7   3919  560  1.55  0.179
Error   39  14084  361
Total   46  18002
S = 19.00   R-Sq = 21.77%   R-Sq(adj) = 7.73%
                      Individual 95% CIs For Mean Based on Pooled StDev of 19.00
Level           N   Mean  StDev  -------+---------+---------+---------+--
Above Hatchet   6  23.82  19.97              (----------*---------)
Barking Kettle  6  30.86  29.28                   (----------*---------)
Beaver Pond     6  29.69   9.44                 (----------*---------)
Broad Cove      6   4.66   5.39  (----------*----------)
Great Pond      6  24.64  34.27               (---------*----------)
Portugal Cove   6  10.92  12.57      (----------*----------)
Split Rock      6  12.28  10.15       (----------*---------)
Watern          5  25.80   3.07               (----------*-----------)
                                    -------+---------+---------+---------+--
                                           0        15        30        45
```

One-way ANOVA: Phospholipids versus Stream, summer

```
Source  DF    SS   MS     F      P
Stream   7  3468  495  3.57  0.005
Error   40  5556  139
Total   47  9024
S = 11.79   R-Sq = 38.43%   R-Sq(adj) = 27.66%
                      Individual 95% CIs For Mean Based on Pooled StDev of 11.79
Level           N   Mean  StDev  ----+---------+---------+---------+-----
Above Hatchet   6   6.86  11.25    (-------*-------)
Barking Kettle  6   9.81  11.91      (-------*-------)
Beaver Pond     6  13.24  11.22         (-------*-------)
Broad Cove      6  28.99  11.49                    (-------*-------)
Great Pond      6  12.62  15.30        (--------*-------)
Portugal Cove   6  26.06  14.65                  (-------*-------)
Split Rock      6   5.49   6.67  (--------*-------)
Watern          6   5.91   9.59   (-------*-------)
                                  ----+---------+---------+---------+-----
                                      0        12        24        36
```

Considering landscape by season separately, hydrocarbons in the spring were significantly higher in forested than in barren landscapes ($p=0.035$). In the summer, free fatty acids were higher in the forested ($p<0.0001$) and AMPL was higher in barren landscapes ($p<0.0001$).

6.3.3 Seston Fatty Acids

Sixty-four fatty acids were identified in the seston samples. Only one replicate in the spring and three replicates in the summer had detectable amounts of fatty acids. A principal component analysis of all fatty acids clearly separated the seasons on PCA1 (Figure 6.7). However, the first three components only explained ~34% of the variance. Thirty-eight of the fatty acids differed significantly ($\alpha=0.05$) by season. PCA1 had a positive weak correlation (~0.2) with long chain fatty acids (22:0, 22:1ω11(13), 21:5ω3, 22:4ω6, 24:1) and negatively with 15:0*i*, and 16:1ω5.

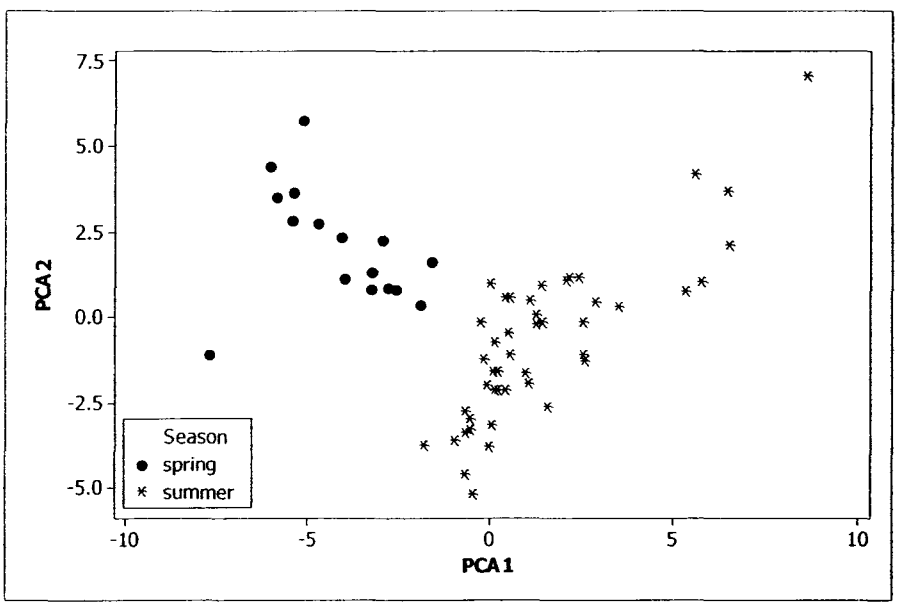

Figure 6.7 Score plot for the seston samples by season using all the fatty acids.

Further analysis used only those fatty acids comprising greater than 1% of the total, which consisted of 24 fatty acids that composed 86% of the total fatty acids identified. The first three components of the principal component analysis only explained 42.8 % of the variance, as shown by the low coefficients used in the axes of the loadings plot (Figure 6.8). There was some differentiation by season on PCA1, with this axis weakly correlating positively with 15:0*i* and 16:1ω5 (~0.4), and negatively with 18:3w3 and 18:1ω9 (~0.3) (Figure 6.9). One-way ANOVAs of the 24 fatty acids showed many of them differed by season, with spring samples having higher proportions of 14:0, 15:0*i*, 16:1ω7, 16:1ω5, 16:4ω3 and 18:4ω3 (Table 6.3). Summer samples had higher 16:0, 16:1ω9, 17:0, 16:3ω4, 18:1ω9, 18:3ω3 and 22:0 (Table 6.3). General trends were not evident among these groups of fatty acids.

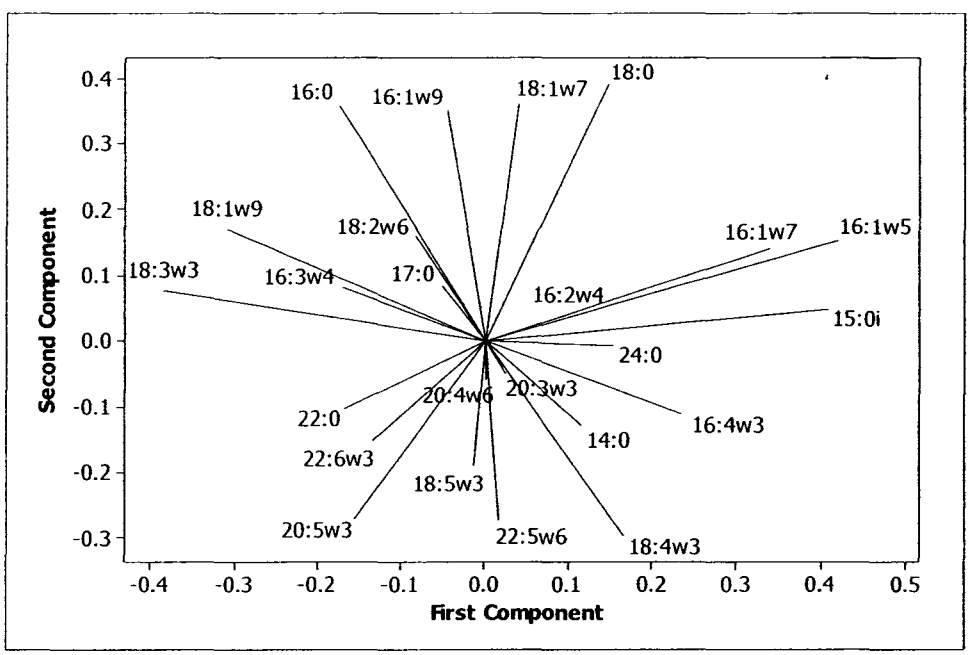

Figure 6.8 Loading plot of seston samples using the 24 fatty acids that were at least 1% of the total.

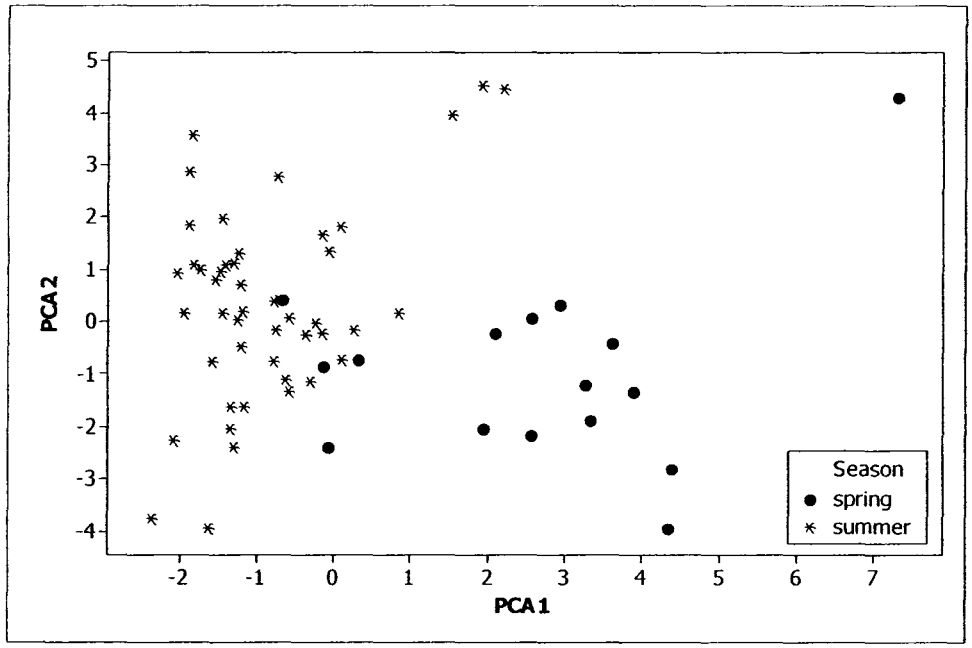

Figure 6.9 Scores plot for seston samples using fatty acids that composed greater than 1% of the total by season.

Table 6.3 Mean and standard deviation (+/-) of the 24 dominant fatty acids by season, with p values given for significant differences between seasons.

	spring		summer		
fatty acid	mean	+/-	mean	+/-	p value
14:0	8.45	2.4	5.94	3.4	0.008
15:0*i*	3.30	2.0	0.48	0.4	<0.0001
16:0	13.82	2.5	17.33	3.5	<0.0001
16:1ω9	0.43	0.4	1.28	1.0	0.002
16:1ω7	7.78	2.6	5.27	2.8	0.002
16:1ω5	2.01	1.4	0.70	0.4	<0.0001
16:2ω4	1.42	0.7	1.53	1.5	ns
17:0	0.08	0.2	1.52	2.7	0.036
16:3ω4	0.87	1.0	2.00	0.9	<0.0001
16:4ω3	4.90	6.8	1.34	1.1	0.001
18:0	3.68	1.9	3.61	1.2	ns
18:1ω9	5.88	3.8	7.87	1.8	0.006
18:1ω7	1.71	1.8	2.03	1.1	ns
18:2ω6	5.42	2.1	4.65	2.3	ns
18:3ω3	3.34	2.1	8.20	2.6	<0.0001
18:4ω3	7.45	3.3	3.53	1.7	<0.0001
18:5ω3	1.65	2.5	1.54	1.3	ns
20:4ω6	1.21	0.6	1.19	0.5	ns
20:3ω3	2.07	5.3	2.18	2.5	ns
20:5ω3	4.26	2.8	5.13	3.9	ns
22:0	0.12	0.2	2.34	3.4	0.013
20:5ω6	1.32	1.1	1.09	1.0	ns
24:0	1.46	2.7	1.35	0.7	ns
22:6ω3	4.16	4.7	3.53	3.5	ns

There was a difference in seston fatty acid composition by season, but it was not attributable to specific groups of fatty acids. This was because of high variability among streams and the low concentration of the spring samples. Fatty acids present in low proportions in the summer samples may have been not detected in the spring samples. Thus only the summer samples were used to further explore differences by location, landscape and stream.

A principal component analysis of the 24 fatty acids (each comprising greater than 1% of the total for the summer samples) showed that the first three components explained ~55% of the variance. Outlets generally scored lower on PCA1 than downstream sites, although this was highly influenced by the three points from Barking Kettle downstream on the far right (Figure 6.10). PCA1 correlated negatively with 18:1ω9 and 18:3ω3 and positively with 15:0*i*, 16:1ω7 and 16:1ω5. Forested streams scored higher on PCA2 which correlated positively with 16:0, 16:1ω9, 18:0 and 18:1ω7. Barren streams scored lower on PCA2 which correlated negatively with 18:4ω3, 20:5ω3 and 22:5ω6. There was some division among streams with the six samples taken from a stream generally clustering for all but Barking Kettle and Portugal Cove, although outlet and downstream samples were associated with each other for these two streams (Figure 6.9). Beaver Pond generally scored higher on PCA2 than Split Rock and Portugal Cove. Broad Cove also scored higher on PCA2 which separated it from Great Pond (Figure 6.9). These differences were slight and were not attributable to a group of fatty acids, thus the fatty acid composition among streams was similar.

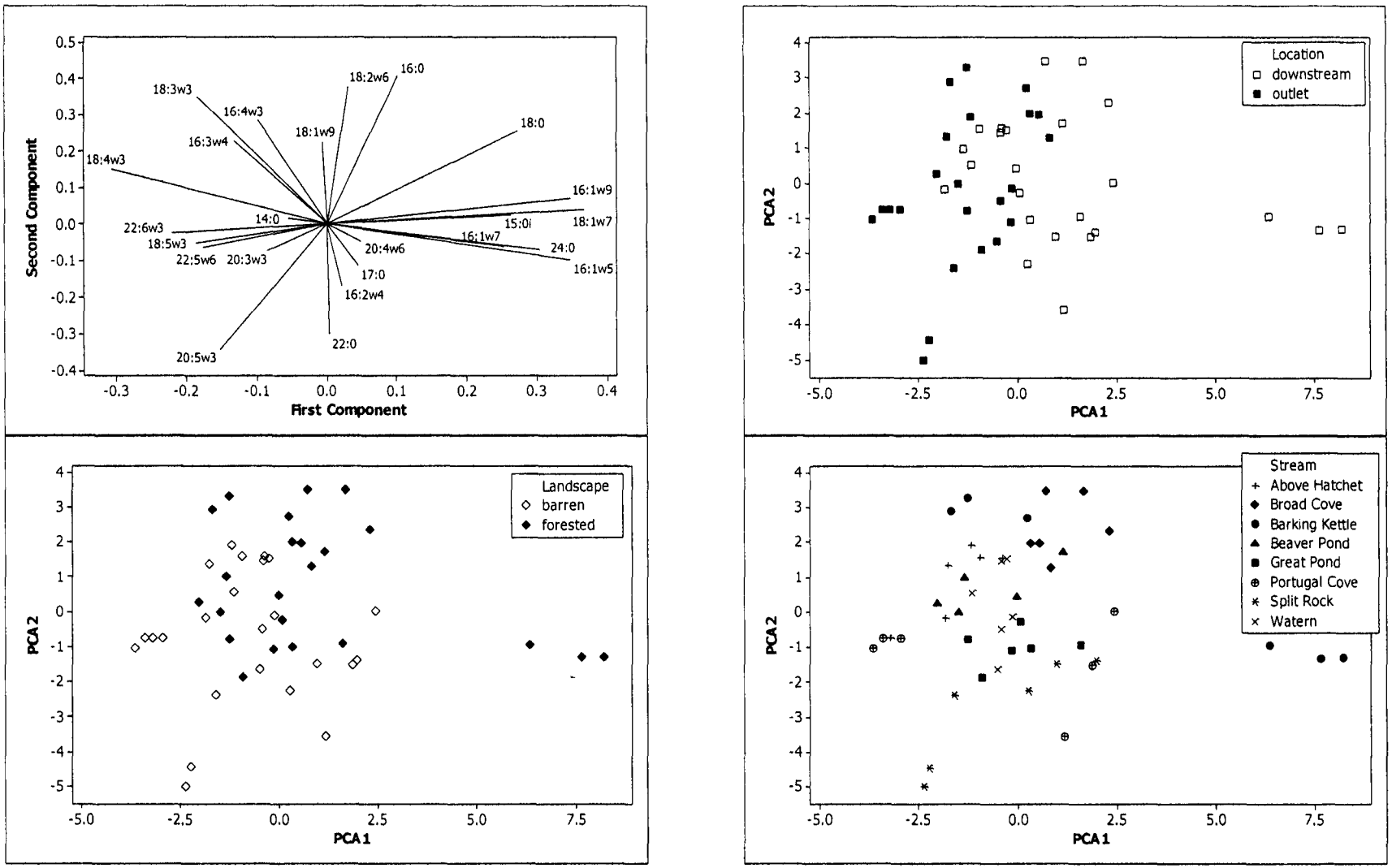

Figure 6.10 Loadings and score plots coded by location, landscape and stream for the summer samples using the 24 dominant fatty acids.

Summer samples were considered separately by location and by landscape using the 24 dominant fatty acids and fatty acid markers (see Appendix 4 and Chapter 5). Carnivory fatty acid markers are so named here to follow the convention used in Chapter 5, where they were an indicator of animal material in the diet of hydropsychids. In this chapter, carnivory fatty acid markers refer to the animal portion of the seston. Outlets had higher proportions of 18:4ω3, 20:5ω3, 22:5ω6 and 22:6ω3. This indicated that in general algal proportions were higher at outlets compared to downstream sites, especially for diatoms containing 20:5ω3, dinoflagellates and green algae (Table 6.4 & Table 6.5). Outlets also had higher proportions of the two carnivory markers and the essential fatty acids (Table 6.5). Downstream sites had more bacteria and cyanobacteria markers than outlets (Table 6.5). Barren streams had more of the general algae, dinoflagellate and green algae markers compared to forested streams (Table 6.5). They also had higher proportions of the two carnivory markers and the long chain essential fatty acids (Table 6.5).

Table 6.4 Mean values of the 24 dominant fatty acids by location and landscape with p values given for significant differences. Bolded values were significantly higher.

fatty acid	Outlet	Downstream	p	Forested	Barren	p
14:0	6.26	5.61	ns	6.38	5.50	ns
15:0i	0.38	0.58	ns	0.56	0.40	ns
16:0	17.08	17.59	ns	**18.82**	15.84	0.002
16:1ω9	0.87	**1.69**	0.003	**1.67**	0.89	0.005
16:1ω7	4.65	5.89	ns	**6.66**	3.88	<0.0001
16:1ω5	0.49	**0.91**	<0.0001	0.74	0.66	ns
16:2ω4	1.17	1.90	ns	1.25	1.82	ns
17:0	0.90	2.14	ns	0.81	2.23	ns
16:3ω4	1.76	2.24	ns	2.10	1.90	ns
16:4ω3	1.60	1.08	ns	**1.73**	0.94	0.013
18:0	3.16	**4.06**	0.011	**3.96**	3.25	0.049
18:1ω9	7.88	7.86	ns	7.54	8.20	ns
18:1ω7	1.53	**2.53**	0.001	2.29	1.76	ns
18:2ω6	4.16	5.14	ns	5.09	4.21	ns
18:3ω3	8.48	7.93	ns	8.78	7.62	ns
18:4ω3	**4.56**	2.50	<0.0001	3.38	3.68	ns
18:5ω3	1.85	1.23	ns	0.85	**2.22**	<0.0001
20:4ω6	1.31	1.08	ns	**1.40**	0.99	0.001
20:3ω3	1.96	2.40	ns	0.76	**3.59**	<0.0001
20:5ω3	**6.28**	3.98	0.040	4.13	6.14	ns
22:0	2.43	2.24	ns	1.49	3.18	ns
22:5ω6	**1.61**	0.56	<0.0001	1.16	1.01	ns
24:0	1.01	**1.69**	0.001	1.46	1.24	ns
22:6ω3	**5.07**	2.00	0.001	2.05	**5.01**	0.002

Table 6.5 Mean values of fatty acid markers for the summer samples using all streams by location, and by landscape with p values for significant differences. Bold text indicates the higher value for significant differences.

Fatty acid marker		Outlet	Downstream	p	Forested	Barren	p
General algae	PUFA	**45.55**	37.44	0.001	38.27	**44.72**	0.007
	sum ω3	**31.96**	22.78	<0.0001	23.65	**31.10**	0.001
Diatoms	20:5ω3	**6.28**	3.98	0.040	4.13	6.14	ns
	$\Sigma C_{16}/\Sigma C_{18}$	0.87	**1.00**	0.046	**1.03**	0.83	0.002
	Leveille diatoms	0.83	0.93	ns	0.91	0.85	ns
Dinoflagellates	22:6ω3	**5.07**	2.00	0.001	2.05	**5.01**	0.002
	$\Sigma C_{18} + \Sigma C_{22}$ PUFA	**28.33**	21.98	0.001	24.36	25.95	ns
Green	18:3ω3	8.48	7.93	ns	8.78	7.62	ns
	ω3/ω6	**4.08**	2.75	0.006	2.50	**4.33**	<0.0001
Golden brown	16:0+18:1ω9	24.95	25.45	ns	26.36	24.05	ns
Cyanobacteria	18:1ω7	1.53	**2.53**	0.001	2.29	1.76	ns
Bacteria	sum (see text)	4.20	**7.08**	0.014	4.94	6.34	ns
Terrestrial	18:3ω3+18:2ω6	12.63	13.07	ns	13.87	11.83	ns
Carnivory	18:1ω9/(16:1ω7+16:1ω7)	**1.50**	1.12	0.027	1.03	**1.58**	0.001
	PUFA/SAFA (P/S)	**1.46**	1.14	0.003	1.18	**1.42**	0.023
all essential	sum (see text)	**25.77**	20.49	0.001	21.99	24.27	ns
essential hufa	sum (see text)	**13.13**	7.42	<0.0001	8.12	**12.43**	0.003

Summer samples for each stream were considered separately by location, outlet (n=3) versus downstream (n=3), for the 24 dominant fatty acids and for the fatty acid markers (see Appendix 4 and Chapter 5). Barking Kettle (Table 6.5) and Portugal Cove (Table 6.6) showed the greatest number of differences between outlets and downstream sites. Barking Kettle outlet had higher proportions of markers for general algae, dinoflagellates, green algae and terrestrial material as well as both carnivory markers and essential fatty acids (Table 6.8). Thus the outlet had a much richer seston composition than the downstream site which had higher proportions of bacteria, cyanobacteria and two of the diatom markers. A similar result was found for Portugal Cove which had higher proportions of the markers for general algae, 20:5ω3, dinoflagellates and green algae as well as both carnivory and essential fatty acid markers. This again demonstrates that Portugal Cove outlet had a much richer seston composition than its downstream sites where markers for cyanobacteria and some diatoms were elevated (Table 6.9). This was a large stream and so changes were expected. However, Beaver Pond was also a large stream which showed fewer changes by location (Table 6.5). Its outlet had higher proportions of 20:5ω3. 22:6ω3 and the long chain essential fatty acids. Beaver Pond differed from all the other streams in that its downstream site had higher proportions of one of the carnivory markers (Table 6.8).

Broad Cove again had a rich and diverse seston composition at its outlet which had higher proportions of markers for dinoflagellates, green algae, one carnivory marker and the longer chain essential fatty acids. Downstream only had higher proportions of the marker for terrestrial material which indicates the influx of this material along the length

of the section sampled (Table 6.8). This was in contrast to Great Pond which showed few changes in seston composition between the outlet and downstream site (Table 6.5). Its outlet had higher proportions of 20:5ω3 and long chain essential fatty acids, whereas downstream only one of the diatom markers was higher (Table 6.8).

Split Rock showed higher proportions of the markers for general algae and carnivory at its outlet, whereas downstream had higher proportions of markers for bacteria and cyanobacteria (Table 6.9). Watern showed few conclusive trends, with markers for dinoflagellates and green algae being higher at the outlet and different markers for these same groups being higher downstream. This also held true for essential fatty acids, with longer chain ones higher at the outlet and shorter chain ones higher downstream. The marker for terrestrial material was higher downstream, and one of the diatom markers was slightly higher at the outlet (Table 6.9). Above Hatchet showed the fewest differences by location of all the streams (Table 6.6), having only slightly higher proportions of a dinoflagellate marker at the outlet (Table 6.9).

Overall, outlets did have higher proportions of a great variety of seston types including general algae, diatoms, dinoflagellates, carnivory markers and essential fatty acids, whereas downstream sites tended to have higher proportions of markers for terrestrial material, bacteria and cyanobacteria. Thus outlets generally had a seston composition that could be considered richer in terms of high-quality food than those at downstream sites. However, there was great variation among streams which could affect the composition of primary and of secondary consumers in these streams.

Table 6.6 Mean values for the 24 dominant fatty acids for forested streams in the summer by location, with p values for significant differences. Bolded values were significantly higher.

	Broad Cove			Barking Kettle			Great Pond			Beaver Pond		
fatty acid	outlet	downstream	p	outlet	downstream	p	outlet	downstream	p	outlet	downstream	p
14:0	**5.54**	4.59	0.020	3.78	**5.58**	0.015	6.72	12.18	ns	**7.96**	4.69	0.005
15:0i	1.04	0.56	ns	0.38	**1.17**	0.016	0.22	0.27	ns	0.30	**0.56**	0.032
16:0	18.13	23.10	ns	20.21	18.63	ns	16.82	17.86	ns	17.82	18.02	ns
16:1ω9	1.43	1.52	ns	1.56	**4.71**	<0.0001	0.71	**1.04**	0.012	0.60	**1.82**	0.003
16:1ω7	3.66	6.24	ns	3.15	**11.61**	0.005	**10.27**	7.38	0.001	**7.15**	3.82	0.001
16:1ω5	0.42	**0.74**	0.039	0.37	**2.03**	0.006	0.54	0.76	ns	0.52	0.54	ns
16:2ω4	0.49	0.77	ns	0.63	**1.59**	0.001	2.01	2.59	ns	1.04	0.85	ns
17:0	0.91	0.81	ns	0.92	1.16	ns	0.51	0.73	ns	0.70	0.73	ns
16:3ω4	1.14	**3.50**	0.006	**1.67**	0.76	0.012	2.24	2.87	ns	2.03	2.58	ns
16:4ω3	2.26	1.77	ns	**4.51**	0.35	0.027	0.76	0.42	ns	1.99	1.81	ns
18:0	5.38	5.03	ns	3.20	**5.45**	0.037	2.54	3.15	ns	2.68	**4.24**	0.020
18:1ω9	**9.76**	7.58	0.048	8.50	7.18	ns	**7.72**	5.22	0.013	5.69	**8.63**	0.011
18:1ω7	2.06	2.73	ns	1.81	**5.43**	0.013	1.27	1.74	ns	1.51	1.77	ns
18:2ω6	6.48	9.47	ns	**7.26**	3.02	0.013	2.45	3.47	ns	4.33	4.23	ns
18:3ω3	9.74	11.37	ns	**12.67**	2.33	<0.0001	7.45	7.38	ns	9.77	9.56	ns
18:4ω3	**4.23**	1.82	<0.0001	**4.77**	0.87	0.013	**3.68**	2.19	0.013	**6.07**	3.37	0.009
18:5ω3	**0.61**	0	0.041	0.79	0.99	ns	0.61	0.54	ns	0.93	2.34	ns
20:4ω6	**1.79**	1.13	0.044	0.89	1.34	ns	1.64	1.40	ns	**2.24**	0.78	<0.0001
20:3ω3	0.27	0.64	ns	0.39	0.73	ns	2.26	0.95	ns	0.20	0.64	ns
20:5ω3	**3.83**	2.59	0.015	1.43	1.48	ns	**9.75**	4.65	0.015	**5.82**	3.46	<0.0001
22:0	1.21	1.21	ns	1.44	**3.08**	0.049	0.80	1.05	ns	0.94	**2.20**	0.036
22:5ω6	**1.66**	0.31	0.002	**1.28**	0.06	0.003	1.30	0.30	ns	2.64	1.74	ns
24:0	1.54	1.34	ns	1.18	2.88	ns	0.84	**1.77**	0.016	0.59	**1.52**	0.003
22:6ω3	**4.33**	0.69	0.002	**1.03**	0.40	0.022	**2.65**	1.34	0.018	**4.94**	1.04	<0.0001

Table 6.7 Mean values for the 24 dominant fatty acids for barren streams at outlets and downstream locations, with p values for significant differences.

	Split Rock			Above Hatchet			Watern			Portugal Cove		
fatty acid	outlet	downstream	p	outlet	downstream	p	outlet	downstream	p	outlet	downstream	p
14:0	2.71	3.32	ns	7.00	5.55	ns	**7.75**	4.94	0.003	**8.64**	4.06	0.001
15:0i	0.24	0.78	ns	0.26	0.35	ns	0.37	0.33	ns	0.24	**0.65**	<0.0001
16:0	9.95	13.54	ns	18.68	18.30	ns	17.60	16.1112	ns	17.39	15.18	ns
16:1ω9	0.41	**0.91**	0.024	0.96	1.32	ns	0.76	0.84	ns	0.57	**1.35**	0.007
16:1ω7	1.99	**4.16**	0.004	2.92	3.15	ns	4.67	**4.96**	0.011	3.35	**5.82**	0.022
16:1ω5	0.33	**0.74**	0.018	0.39	0.58	ns	1.00	0.82	ns	0.34	**1.09**	0.004
16:2ω4	0.45	0.54	ns	1.50	2.47	ns	1.93	2.09	ns	1.27	4.32	ns
17:0	0.28	0.40	ns	1.19	2.55	ns	1.52	1.41	ns	1.18	9.36	ns
16:3ω4	1.21	1.26	ns	3.12	3.17	ns	1.00	**2.33**	0.009	1.69	1.44	ns
16:4ω3	**1.12**	0.63	0.015	0.97	1.53	ns	0.59	1.14	ns	0.58	**0.99**	0.006
18:0	1.94	**3.59**	0.026	3.42	3.76	ns	3.83	3.51	ns	2.27	3.72	ns
18:1ω9	6.50	8.75	ns	8.45	10.32	ns	8.48	8.63	ns	7.93	6.59	ns
18:1ω7	0.99	**2.37**	0.001	1.45	1.81	ns	2.08	1.92	ns	1.03	**2.44**	0.008
18:2ω6	2.20	**4.07**	0.019	4.20	3.95	ns	3.32	**8.99**	0.005	3.04	3.95	ns
18:3ω3	7.75	7.49	ns	8.43	8.68	ns	6.19	**10.91**	0.002	5.82	5.71	ns
18:4ω3	**2.49**	1.32	0.003	**5.38**	4.53	0.032	3.81	3.88	ns	**6.06**	1.99	<0.0001
18:5ω3	**1.40**	0.59	0.004	**3.11**	2.48	0.037	2.65	1.66	ns	**4.67**	1.22	<0.0001
20:4ω6	1.27	1.06	ns	0.69	0.84	ns	1.35	1.16	ns	0.64	**0.89**	0.005
20:3ω3	0.43	0.35	ns	4.83	5.80	ns	4.48	4.01	ns	2.79	6.04	ns
20:5ω3	16.05	9.72	ns	3.60	3.41	ns	3.34	3.68	ns	**6.43**	2.87	0.002
22:0	13.07	7.89	ns	0.69	0.45	ns	0.85	0.98	ns	0.45	**1.08**	0.002
22:5ω6	2.53	0.58	ns	0.67	0.44	ns	**1.99**	0.81	0.004	**0.83**	0.26	0.044
24:0	1.49	1.78	ns	0.90	1.21	ns	0.90	0.99	ns	0.64	**1.98**	0.018
22:6ω3	1.70	1.59	ns	6.04	5.21	ns	**5.08**	3.12	0.001	**14.76**	2.59	<0.0001

Table 6.8 Mean values of fatty acid markers for the summer samples of forested streams by location, with p values for significant differences. Bold text indicates the higher value for significant differences.

Fatty acid marker		Broad Cove			Barking Kettle			Great Pond			Beaver Pond		
		outlet	downstream	p	outlet	downstream	p	outlet	downstream	p	outlet	downstream	p
General algae	PUFA	40.30	36.57	ns	**45.62**	16.33	0.001	42.20	35.02	ns	46.33	41.79	ns
	sum ω3	26.35	19.06	0.003	**30.23**	7.89	0.003	28.42	19.20	ns	31.49	26.54	ns
Diatoms	20:5ω3	3.83	2.59	0.015	1.43	1.48	ns	**9.75**	4.65	0.015	**5.82**	3.46	<0.0001
	$\Sigma C_{16}/\Sigma C_{18}$	0.71	0.98	0.002	0.82	**1.46**	0.003	1.23	1.17	ns	1.00	0.86	0.025
	Leveille diatoms	0.61	0.67	ns	0.47	**1.07**	0.019	1.28	**1.43**	0.018	1.03	0.70	ns
Dinoflagellates	22:6ω3	**4.33**	0.89	0.003	**1.03**	0.40	0.022	2.65	1.34	ns	**4.94**	1.04	<0.0001
	$\Sigma C_{18} + \Sigma C_{22}$ PUFA	**28.52**	25.46	0.028	**32.97**	9.73	<0.0001	21.22	17.95	ns	31.32	27.70	ns
Green	18:3ω3	9.74	11.37	ns	**12.67**	2.33	<0.0001	7.45	7.38	ns	9.77	9.56	ns
	ω3/ω6	**2.34**	1.53	0.010	2.64	1.33	ns	4.08	2.58	ns	2.85	2.67	ns
Golden brown	16:0+18:1ω9	27.89	30.68	ns	28.72	25.81	ns	24.54	23.08	ns	23.51	28.65	ns
Cyanobacteria	18:1ω7	2.06	2.73	ns	1.81	**5.43**	0.013	1.27	1.74	ns	1.51	1.77	ns
Bacteria	sum (see text)	5.69	5.84	ns	4.63	**7.98**	0.033	3.25	4.14	ns	3.38	4.65	ns
Terrestrial	18:3ω3+18:2ω6	16.22	**20.85**	0.003	**19.93**	5.34	<0.0001	9.90	10.84	ns	14.06	13.80	ns
Carnivory	18:1ω9/(16:1ω7+18:1ω7)	**1.73**	0.89	0.023	**1.75**	0.43	0.017	0.67	0.57	ns	0.66	**1.55**	0.001
	PUFA/SAFA (P/S)	1.22	1.00	ns	**1.46**	0.47	0.002	1.44	1.03	ns	1.47	1.31	ns
all essential	sum (see text)	26.37	25.29	ns	**24.96**	8.88	<0.0001	24.11	18.73	ns	27.43	20.19	0.006
essential hufa	sum (see text)	**10.15**	4.44	<0.0001	5.02	3.53	ns	**14.21**	7.89	0.032	**13.33**	6.39	<0.0001

Table 6.9 Mean values of fatty acid markers for the summer samples of barren streams by location, with p values for significant differences. Bold text indicates the higher value for significant differences.

Fatty acid marker		Split Rock			Above Hatchet			Watern			Portugal Cove		
		outlet	downstream	p	outlet	downstream	p	outlet	downstream	p	outlet	downstream	p
General algae	PUFA	**52.19**	40.46	0.003	46.05	34.59	ns	41.19	47.87	ns	**50.53**	34.88	0.01
	sum ω3	**35.96**	26.57	0.019	33.55	32.23	ns	28.13	29.04	ns	**41.58**	21.73	0.00
Diatoms	20:5ω3	16.05	9.72	ns	3.60	3.41	ns	3.34	3.68	ns	**6.43**	2.87	0.00
	$\Sigma C_{16}/\Sigma C_{18}$	0.66	0.77	ns	0.82	0.87	ns	**0.88**	0.71	0.014	0.80	**1.16**	0.02
	Leveille diatoms	0.66	0.70	ns	0.85	0.81	ns	0.89	0.91	ns	0.88	1.10	ns
Dinoflagellates	22:6ω3	1.70	1.58	ns	6.04	5.21	ns	**5.08**	3.12	0.001	**14.76**	2.59	<0.0001
	$\Sigma C_{18} + \Sigma C_{22}$ PUFA	21.86	21.27	ns	**29.46**	26.32	0.022	25.21	**30.52**	0.008	**36.09**	16.85	<0.0001
Green	18:3ω3	7.75	7.49	ns	8.43	8.68	ns	6.19	**10.91**	0.002	5.82	5.71	ns
	ω3/ω6	4.49	2.80	ns	5.06	5.31	ns	**3.48**	2.30	0.003	**7.71**	3.49	0.003
Golden brown	16:0+18:1ω9	16.45	22.29	ns	27.13	28.62	ns	26.07	24.74	ns	25.32	21.77	ns
Cyanobacteria	18:1ω7	0.99	**2.37**	0.001	1.45	1.81	ns	2.08	1.92	ns	1.03	**2.44**	0.008
Bacteria	sum (see text)	1.91	**4.26**	0.015	4.71	6.44	ns	6.46	6.04	ns	3.58	17.28	ns
Terrestrial	18:3ω3+18:2ω6	9.95	11.56	ns	12.64	12.63	ns	9.50	**19.89**	0.001	8.86	9.65	ns
Carnivory	18:1ω9/(16:1ω7+18:1ω7)	**2.17**	1.35	0.016	1.93	2.09	ns	1.26	1.25	ns	**1.82**	0.79	0.002
	PUFA/SAFA (P/S)	**1.71**	1.24	0.012	1.47	1.40	ns	1.24	1.70	ns	**1.64**	0.97	0.016
all essential	sum (see text)	29.76	24.39	ns	23.06	22.57	ns	19.66	**27.90**	0.001	**30.79**	16.00	0.001
essential hufa	sum (see text)	19.81	12.83	ns	10.43	9.94	ns	**10.15**	8.01	<0.0001	**21.94**	6.35	<0.0001

6.4 Discussion

6.4.1 Seston Lipid Classes

Differences among some seston lipid classes by season were evident, with spring samples having higher proportions of TAG, sterols and diacylglycerols whereas summer samples had higher proportions of free fatty acids and AMPL. TAG is a storage lipid, regulated by nutrient availability which may have been more plentiful in the spring because of increased runoff and higher discharge levels (Parrish 1988). This relation to nutrient flux was supported by Parrish et al. (2000) who found higher levels in the spring in nutrient rich oceanic upwelling regions. Sterols are used in membrane structure, regulating fluidity, and so would have higher levels when organisms are growing (Parrish et al. 2000). AMPL indicated more plant material in the seston in the summer as this class consists of pigments and glycolipids associated with chloroplasts (Arts et al. 1997; Parrish et al. 2000). AMPL was higher in barren streams in the summer, presumably because of increased solar radiation causing increased plant growth compared to forested streams.

Free fatty acids are produced when lipids break down. Higher levels in summer possibly resulted from spring algal blooms decaying in the lake and warmer conditions during sample transport causing more degradation (Arts et al. 1997; Parrish et al. 2000). They were also higher in forested streams which are warmer than barren streams (Chapter 4) which may have increased their rate of degradation.

Kreeger et al. (1997) found an inverse relationship between TAG and PL of lake seston, with TAG concentrations low from February to April and peaking in November,

while PL concentrations were high from February to April and low in November. This relationship was not seen here, but since samples were not collected consistently over the year, long term seasonal trends cannot be examined. Kreeger et al. (1997) also found that as lakes became more eutrophic, TAG decreased and PL increased. Similar trends were not seen here. TAG was highest in Broad Cove and Great Pond whose lakes had septic tank beds surrounding them whereas Watern and Above Hatchet were in barren landscapes without any developments and had lower proportions of TAG. Trends in PL proportions were also not evident. Lakes were not classified in terms of their trophic status for the purposes of this study, but observations do not appear to agree with the results of Kreeger et al. (1997).

When seasons were considered separately, lipid classes also differed among streams. In the spring, Great Pond had high levels of TAG (Table 6.2). This stream had higher abundances of phytoplankton than other streams (Chapter 4) and this may account for higher levels of TAG if there was a spring phytoplankton bloom. Free fatty acid levels were higher in Great Pond and Broad Cove, both rich streams which had elevated TAG levels. This may indicate more cell degradation in streams rich in phytoplankton (Chapter 4). There was more variation in free fatty acid levels among streams in the summer which could again be linked to temperature or the amount of organic matter in the sample. Hydrocarbons also differed among streams in the summer with Split Rock having the greatest proportion. Sources of hydrocarbons include petroleum spills as well as alkanes in algae or plant leaves (Parrish et al. 2000). Split Rock runs under a major electrical transmission line with ATV/snowmobile service travel and through an area of cabins with

recreational ATV/snowmobile trails and so this stream may have received fuel spills. Broad Cove, Great Pond and Beaver Pond were crossed by roads, but had lower proportions of hydrocarbons. Above Hatchet and Watern had elevated proportions of AMPL in the summer, suggesting high levels of pigmented plankton (Parrish et al. 2000). Broad Cove and Portugal Cove were the only two streams to show an increase in PL levels from the spring to the summer samples, and since PL is used in membrane structure these samples may have contained cast off exoskeletons or other such material.

Using PCA analysis, seston lipid classes were not clearly distinct by season, location or landscape, although spring samples did show more variability (Figure 6.6). Low concentrations of lipids in spring samples required a much larger volume of material to be spotted on the iatroscan rods, which reduced precision, although standard errors are similar for both seasons. Standard deviations for both seasons were large, which could result from a patchy seston composition and abundance within and among streams.

6.4.2 Seston Fatty Acids

Only one other study was found describing the fatty acid composition of river seston (Acharya et al. 2005). Values were reported as μg per mg dry weight carbon and so were not directly comparable to these results. Fatty acids reported to have the greatest weight in river seston were 16:0, 20:5ω3, 21:0, 18:0 and 16:1 (Acharya et al. 2005). In the current study 16:0 was the fatty acid in the greatest proportion, and 16:1ω7 had the fifth highest proportion in the summer samples, similar to that of Acharya et al. (2005).

Other studies investigated lake phytoplankton and zooplankton fatty acid composition (Ahlgren et al. 1997; Ahlgren et al. 1992; Bourdier & Amblard 1988; Brett

& Muller-Navarra 1997; Kainz et al. 2004; Leveille et al. 1997; Sekino et al. 1997; Sushchik et al. 2004). Ahlgren et al. (1992) showed that fatty acid composition differed among groups of freshwater algae. Most of their samples were from lab cultures with some from lake net-tows. This allowed characterization of differences among groups which aided the development of fatty acid markers. However, fatty acid composition of a species can differ between the lab and the natural environment (Sushchik et al. 2004). Leveille et al. (1997) determined the fatty acid composition of phytoplankton samples from a lake which exhibited successive population changes, and developed fatty acid markers for this natural community which provided some agreement with other markers for the same algal groups in the current study. Thus there is evidence that fatty acid composition can be used as a tool for assessing changes in the phytoplankton composition in freshwater lakes.

Environmental conditions (light, temperature, nutrients) will cause phytoplankton to have a highly variable lipid class and fatty acid composition depending on the species present (Ahlgren et al. 1997; Sushchik et al. 2004). Thus changes in environmental conditions could explain some of the variation among and within the streams seen here. Non-optimal light levels stress phytoplankton cells, causing increases in lipid content, mostly by increasing the saturated and ω6 fatty acids resulting in a decrease in the proportion of ω3 fatty acids. This reduces the quality of the phytoplankton because ω3 fatty acids are essential to higher trophic levels since they are only produced by plants and so must be obtained from food (Ahlgren et al. 1992). Phytoplankton had higher abundances of chlorophyll-a in barren streams (Chapter 4) which may indicate increased

availability of light. Higher temperatures can also cause the proportion of PUFA to decrease (Sushchik et al. 2004). Here, water temperature was higher at outlets than downstream (Chapter 4) but proportions of PUFA were higher at outlets because of the outflow of lake phytoplankton, thus there is a potential interaction of factors.

Nutrient limitation can also alter the fatty acid composition of phytoplankton. Low levels of nitrogen cause a reduction in amino acid synthesis which results in an increased production of lipids, an affect which has been demonstrated in diatoms (Groeger & Kimmel 1988). Moderate nitrogen deficiencies can cause increased production of nitrogen fixing cyanobacteria (Arts et al. 1997). Ahlgren et al. (1997) found a positive relationship between phosphorous concentrations and the ω3 PUFA content of lake phytoplankton. Low levels of nitrogen and phosphorous caused green algae to develop thicker cell walls as a defensive mechanism against herbivore consumption during periods of stress (Van Donk et al. 1997). In these cases, fatty acid markers would not correctly identify algal groups (Dalsgaard et al. 2003). Nutrient levels were not measured in the streams in the current study, but forested soils do have higher nutrient concentrations than barren soils (Heringa 1981).

Food quality is related to the PUFA content of the seston (Ahlgren et al. 1997). Seston here can generally be considered a high quality food source as the proportion of PUFA was high (~40%), consisting mostly of ω3 PUFA (~30%). Outlets had higher PUFA and ω3 PUFA proportions than downstream sites, as did barren compared to forested landscapes. Barking Kettle downstream had the lowest proportions of PUFA and

ω3 PUFA compared to all other sites. Blackflies emerging from this site were very small with a low fecundity, evidence for a lower food quality at this site (Colbo 1982).

Phytoplankton low in ω3 PUFA also negatively affects growth rates and egg production of zooplankton such as the cladocerans *Daphnia* and *Bosmina* (Acharya et al. 2005; Muller-Navarra et al. 2000). Acharya et al. (2005) fed *Bosmina* river seston and found lower growth rates and egg production than when fed a unialgal culture of *Scenedesmus acutus*, a green alga. Similarly, Muller-Navarra et al. (2000) found lower 20:5ω3 content limited the production of *Daphnia*. They sampled a series of 13 lakes and found that total phosphorous concentrations and cyanobacteria production increased as lakes became more eutrophic. Cyanobacteria have low levels of ω3 PUFA and so cannot support zooplankton production. Therefore *Daphnia* growth rates and production decrease with increasing total phosphorous concentrations of lakes (Muller-Navarra et al. 2004). Newfoundland streams also contain lake zooplankton (Chapter 3 & 4) and so the above factors would play a role here. More basic research on the fatty acid composition of stream zooplankton is needed before it can be accurately detected in seston samples and robust fatty acid markers developed.

Much of the literature focuses on essential fatty acids (18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3 and 22:6ω3) because they are required for growth, reproduction and general physiology of organisms (Arts et al. 2001). Unfortunately values were reported as proportions of dry weights and so cannot be directly compared to those measured here (Brett & Muller-Navarra 1997; Kainz et al. 2004). However, relative proportions of these fatty acids to each other in the literature seem comparable to those found in seston here.

Seasonal changes in lake phytoplankton fatty acid composition caused by successive changes in the dominant phytoplankton taxa have been reported by Suschik et al. (2004) and Ahlgren et al. (1997) and observed here. Summer samples had greater proportions of long chain fatty acids, some of which are associated with zooplankton (22:0, 22:1ω11(13)) and terrestrial material (22:0). Spring samples had higher proportions of a bacterial fatty acid (15:0*i*). Usually these fatty acids are accompanied by other indicative fatty acids but since this was not the case here further speculation as to the cause of the seasonal separation is not possible. Using only the 24 dominant fatty acids in a PCA also showed a seasonal separation, with spring samples again having higher proportions of 15:0*i*. Summer samples had more 18:3ω3, indicative of green plant material that could be more plentiful in summer as it is a time of high primary production.

Consideration of only the summer samples showed outlets had slightly higher proportions of 15:0i (bacteria), 16:1ω7 and 16:1ω5, while downstream samples had slightly more 18:1ω9 and 18:3ω3 (green plants). Forested streams correlated with fatty acids 16:0, 16:1ω9, 18:0 and 18:1ω7 which are the start of the pathway of fatty acid synthesis in autotrophic and heterotrophic organisms. Barren streams correlated weakly with fatty acids 18:4ω3, 20:5ω3 and 22:5ω6 which are found near the end of these pathways (Dalsgaard et al. 2003) (Figure 6.10). Streams generally clustered, and for the two streams which did not, their outlet and downstream samples clustered showing there was much less variance among the replicates.

Most streams differed in their fatty acid composition from outlets to downstream. Distances of downstream sampling sites were equivalent to station 8 (Table 4.2). This difference was especially pronounced in Portugal Cove whose outlet had greater proportions of algae, dinoflagellates and carnivory markers. This large stream was sampled 2.5 km downstream from the outlet because seston is carried a longer distance in large streams because of higher discharge. Over such a length one would expect the seston composition to differ because of material settling out of the water and other material entering the stream. However, terrestrial markers did not differ by location in this stream. Portugal Cove has parallel fens that may flush into the stream with rain which would influence the seston fatty acid composition. Beaver Pond was another large stream with few differences in seston composition. Smaller streams showed much variability, with Barking Kettle showing many significant differences between outlet and downstream even though its downstream site was only 110 m from the outlet. However, Above Hatchet was sampled 193 m downstream and showed only two significant differences by location. Overall, the fatty acid composition of streams was highly variable, both among streams and within a stream. This demonstrates the highly changeable seston compositions to which filter feeders need to adapt.

Sites were only sampled twice and so cannot provide a general overview of the seston composition. To gain a more in-depth picture of location, landscape and stream differences more sampling at regular intervals would be required. In addition, the lipid and fatty acid composition of major groups of phytoplankton and zooplankton from different streams needs to be assessed to determine the inter-stream variability. From that

the usefulness of fatty acid markers can be assessed. Note that bacteria were under-represented in samples that were filtered in the field using a 10 μm sieve and so a better field filtration method or a more sensitive analytical method is needed. However, even this brief study shows differences from outlet to downstream, indicating that seston communities differ. Consequently, food available to filter feeding organisms varies depending on the stream, and the location within that stream they inhabit. The fatty acid composition of outlets was consistently different between outlets and downstream sites within a stream, but these differences were not consistent across streams.

This study was only a preliminary indication of the lipid profile of these freshwater lotic systems. However, this research provided an important step forward to a greater understanding of the dynamics of these lacustrine ecosystems and provided a basis for asking directed research questions to extend marine research approaches to freshwaters.

6.5 Conclusion

Characterization of the lipid and fatty acid composition of the seston in lotic ecosystems is an innovative approach to studying the ecology of these habitats. Differences among and within streams indicated the dynamic nature of the seston community. Changes in lipid and fatty acid composition suggested there was an influence of lake seston on the lotic community. It is not known if this change is exploited by filter feeders. If so, it could influence their use of lotic habitats. The next chapter explores the relationship between the seston and the hydropsychid community in terms of their lipid class and fatty acid composition.

7. CHAPTER 7: COMPARISON OF STREAM SESTON LIPID CLASS AND FATTY ACID COMPOSITION WITH THOSE OF LARVAL HYDROPSYCHIDAE

7.1 Introduction

Larval Hydropsychidae filter-feed by building benthic, fixed retreats on stable substrates with a silk net at the opening to sieve seston from flowing waters. One approach to investigate what types of food larvae are utilizing is by analyzing lipids in both the larvae and the seston. However, very little research is known about lipid components of freshwater lotic ecosystems. Some preliminary findings on selected freshwater insects are available (Bell et al. 1994; Cargill et al. 1985; Hanson et al. 1985; Henderson et al. 1996), with even less information available on lotic seston (Acharya et al. 2005).

The first objective of this chapter is to directly explore the relation of the lipid class and fatty acid composition of freshwater seston (Chapter 6) to that of hydropsychids (Chapter 5) collected at the same time. This approach has been used in marine systems, where the lipid composition of plankton net tows was compared to that of secondary consumers (Budge et al. 2001; Copeman & Parrish 2003). A recent study by Acharya et al. (2005), who fed riverine *Bosmina* (Cladocera) seston, suggests that this approach is feasible in lotic ecosystems. A second objective is to expand the analysis of the above relationship between seston and hydropsychids to determine the influence of location, landscape and stream. If the seston lipid class and fatty acid composition does not reflect that of the hydropsychids, it suggests that larvae are selectively feeding from the seston. However, if differences in the lipid class and fatty acid composition of the seston among

sites are reflected in the larvae, then this suggests that larvae are opportunistic feeders, using whatever is available, and therefore have a wide range of possible food types.

7.2 Materials and Methods

Data for this chapter have been reported in Chapter 5 and 6. Detailed materials and methods were given in section 5.2 (study area, collection of Hydropsychidae, lipid extraction and lipid analysis). Collection, extraction and analysis of seston samples were described in section 6.2. Data were entered into Microsoft Excel and analyzed in Minitab 14.2.

7.3 Results

7.3.1 Lipid classes: comparison of seston and Hydropsychidae

For determination of lipid classes, all water samples were assayed (n=48 spring, n=48 summer) as were 67 hydropsychid samples. Not all hydropsychid samples were examined as lipid classes were found not to differ by species or life stage among the samples assayed. Lipid class values were expressed as a percentage of the total so they were comparable among seston and hydropsychid samples. Seston samples consisted mainly of hydrocarbons (HC), triacylglycerols (TAG), acetone mobile polar lipids (AMPL), and phospholipids (PL) with the standard deviations showing a high degree of variability in the data set (Table 7.1). Hydropsychids consisted mainly of TAG, PL and AMPL, but again there was a lot of variability (Table 7.2). Thus lipid class composition generally differed between seston and hydropsychid samples.

Table 7.1 Mean and standard deviation (+/-) of lipid classes from all stream seston samples by season and for both seasons combined.

	spring		summer		both seasons	
Lipid Class	mean	+/-	mean	+/-	mean	+/-
Hydrocarbons	12.23	14.2	14.93	14.2	13.59	14.2
Steryl Esters/Wax Esters	1.44	3.0	1.15	1.7	1.29	2.4
Ethyl Esters	0.12	0.6	0.00	0.0	0.06	0.4
Methyl Esters	0.28	1.7	0.00	0.0	0.14	1.2
Ethyl Ketones	3.61	5.4	0.55	2.1	2.07	4.4
Methyl Ketones	1.83	5.4	0.00	0.0	0.90	3.9
Glycerol Ethers	0.50	3.4	0.00	0.0	0.25	2.4
Triacylglycerols	19.62	16.6	4.84	7.5	12.15	14.8
Free Fatty Acids	5.16	6.2	8.81	10.0	7.01	8.5
Alcohols	3.30	4.5	3.00	3.3	3.15	3.9
Sterols	10.30	7.3	7.60	5.7	8.93	6.6
Diacylglycerols	2.94	5.0	1.03	2.0	1.98	3.9
Acetone Mobile Polar Lipids	30.91	21.6	44.46	19.4	37.76	21.5
Phospholipids	20.22	19.8	13.62	13.9	16.89	17.3

Table 7.2 Mean and standard deviation (+/-) of lipid classes for all hydropsychid samples from both seasons and all streams.

Lipid Class	mean	+/-
Hydrocarbons	2.81	2.8
Steryl Esters/Wax Esters	0.51	2.1
Ethyl Esters	0.04	0.3
Methyl Esters	0.00	0.0
Ethyl Ketones	0.53	1.0
Methyl Ketones	0.24	1.0
Glycerol Ethers	0.00	0.0
Triacylglycerols	65.29	20.0
Free Fatty Acids	6.58	9.5
Alcohols	0.24	0.7
Sterols	1.18	1.6
Diacylglycerols	0.41	1.3
Acetone Mobile Polar Lipids	6.13	11.0
Phospholipids	16.04	12.2

A principal component analysis (PCA) was conducted on the lipid classes of combined seston and hydropsychid data, with the first three components explaining 42.1% of the variance. The seston and hydropsychids separate on PCA1 which was positively correlated with sterols and AMPL (~0.4) and negatively correlated with TAG (Figure 7.1, Figure 7.2). There were no clear distinctions by season, river, location or landscape, nor was it possible to discern these relationships when comparing hydropsychids to seston at a given site.

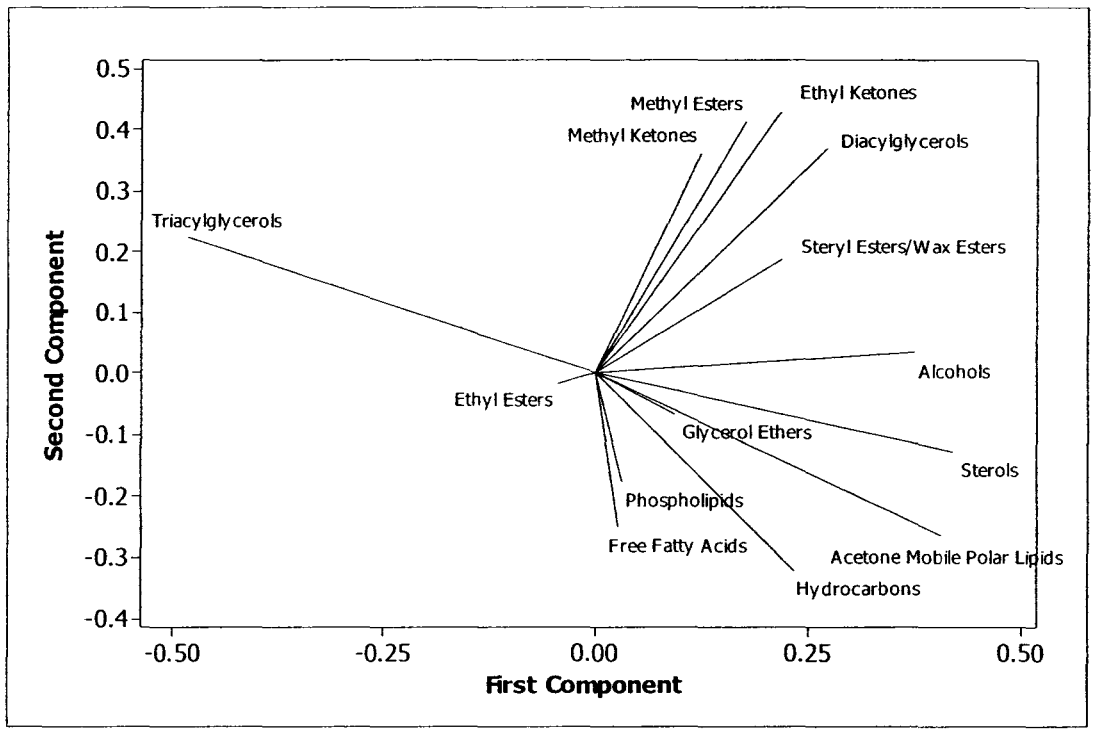

Figure 7.1 Loading plot for the PCA of lipid classes for seston and hydropsychid samples.

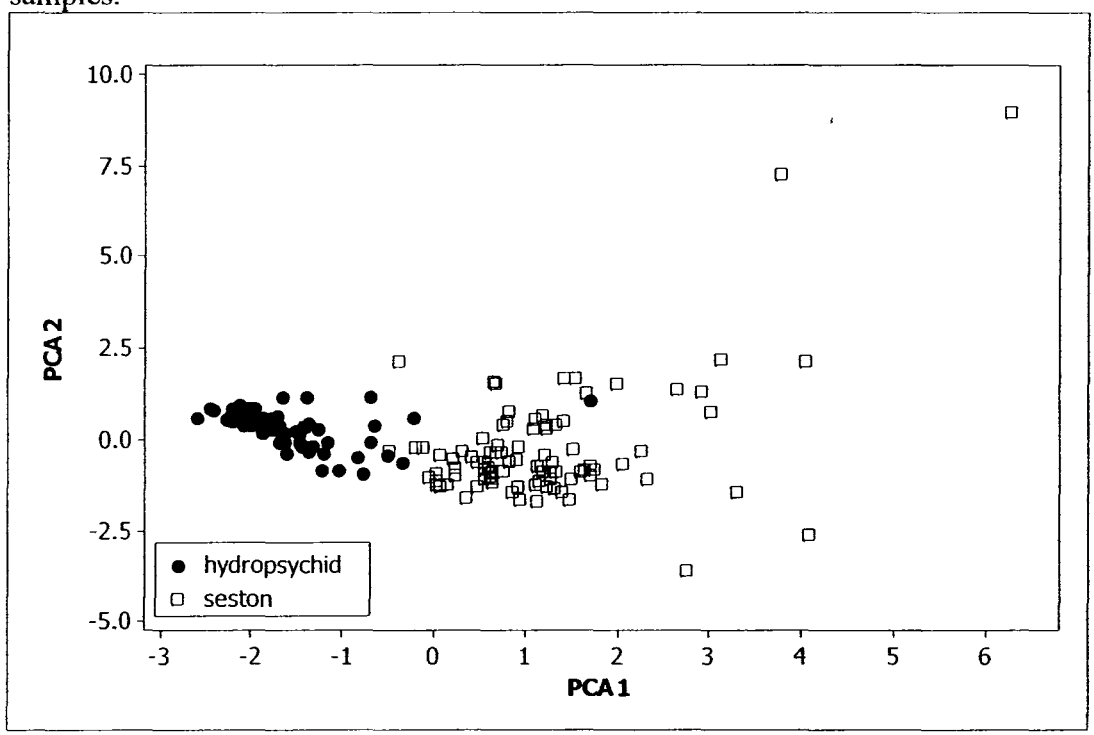

Figure 7.2 Score plot for the PCA of lipid classes for seston and hydropsychid samples.

7.3.2 Fatty acids: comparison of seston and Hydropsychidae

Fatty acid compositions of hydropsychid and seston samples were compared using PCA of all 64 identified fatty acids. The first three components only explained 31.1% of the variance. However, there was a clear distinction between the hydropsychid and the seston fatty acid composition on PCA1, with seston scoring higher on PCA1 (Figure 7.3; loading plot not shown as it was not possible to distinguish the 64 individual fatty acids on the plot). PCA1 was weakly positively correlated (~0.2) with 16:3ω4, 18:5ω3, 23:0, 22:5ω6, 22:4ω3 and 24:0 and weakly negatively correlated (~0.2) with 18:1ω9 and 20:5ω3. Spring samples of both seston and hydropsychids generally scored higher on PCA2 compared to summer samples, although there was a clear seasonal distinction for the seston samples (Figure 7.3). PCA2 was weakly correlated (~0.2) negatively with 22:0, 22:1ω11(13), 22:4ω3 and 24:1 and positively with 15:0*i*, 16:0*i*, 16:0*ai*, 16:1ω5 and 18:1ω11. As there were no strong correlations that definitively separated hydropsychids and seston, their fatty acid composition was similar.

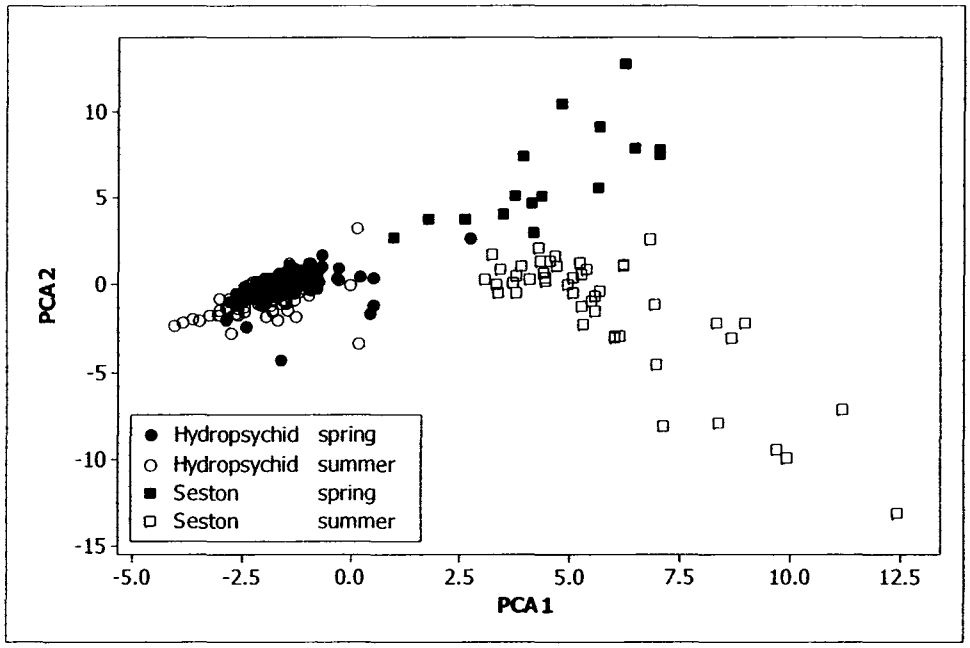

Figure 7.3 Score plot of the 64 fatty acids, coded by season for seston and hydropsychids.

A PCA of the dominant 14 fatty acids, each of which constituted at least 1% of the total fatty acid composition, showed that the first three components explained ~54% of the variance. This is more than when all 64 fatty acids were used. The hydropsychid and seston samples separated (Figure 7.5), but with more scatter than when 64 fatty acids were used (Figure 7.3). The distinction between seasons for either seston or hydropsychids was more blurred, but both had the same directional shift, with spring samples scoring lower on PCA1 and PCA2 compared to summer samples (Figure 7.5). PCA1 was weakly correlated (~0.2 - 0.3) negatively with 14:0, 16:0, 16:1ω7, 18:4ω3 and 22:6ω3 and positively with 16:1ω9, 18:0, 18:1ω9, 18:1ω7, 18:3ω3, 20:4ω6 and 20:5ω3 (Figure 7.4). Thus dominant food resources changed seasonally in the seston and this was reflected in the hydropsychid diet.

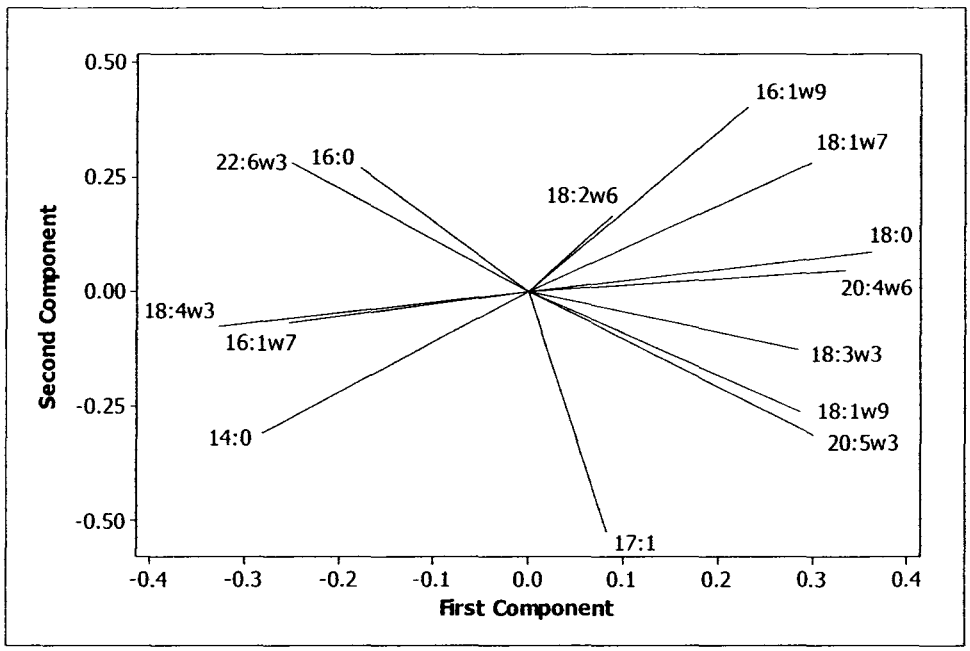

Figure 7.4 Loading plot of the 14 fatty acids comprising at least 1% of the total fatty acid composition.

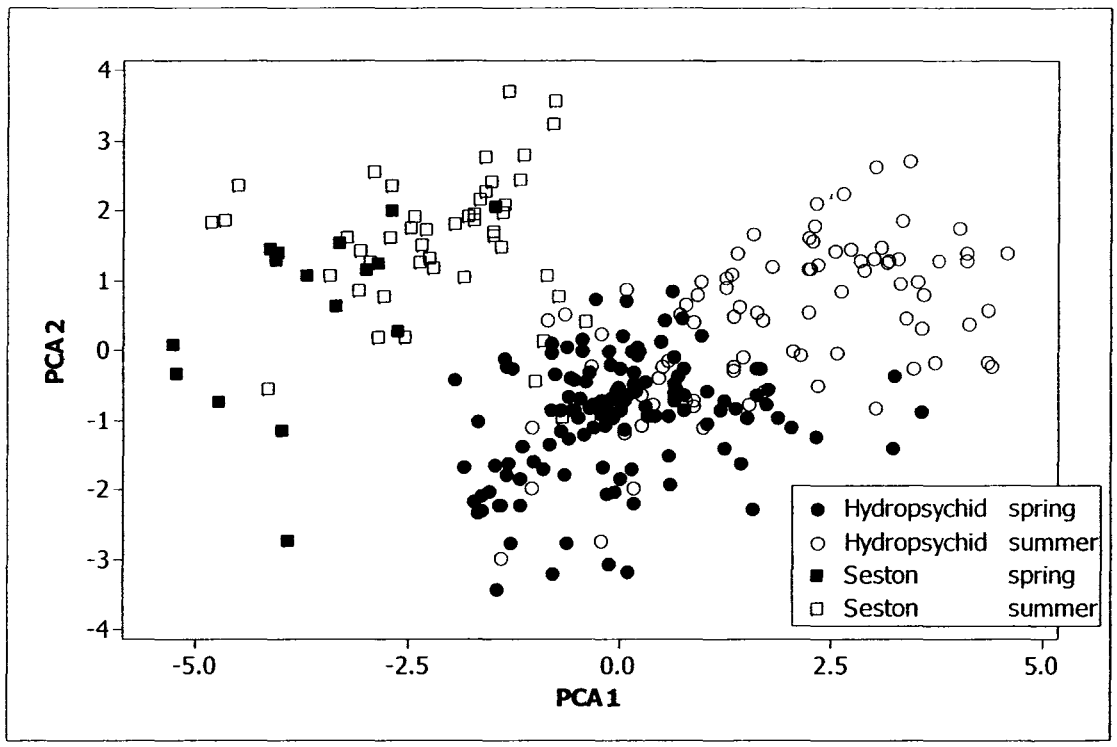

Figure 7.5 Score plot of the 14 fatty acids each comprising at least 1% of the total fatty acid composition, coded by season for seston and hydropsychids.

7.3.3 Fatty acid markers: comparison of seston and Hydropsychidae

Fatty acids reported to be markers for certain groups of organisms (see Appendix 4, Chapters 5 & 6) were analyzed to further investigate the use of this tool to elucidate what components of the seston hydropsychids may be utilizing as food. Differences between fatty acid markers in hydropsychids and seston were analyzed with One-way ANOVAs (Figure 7.6 - Figure 7.9). There were significant differences (α=0.05) between these fatty acid markers by type (Table 7.3).

Because the above results suggested a seasonal shift, differences between hydropsychid and seston markers were considered over spring, summer and both seasons. Overall, seston had higher levels of both dinoflagellate markers and of two of the three diatoms markers than the hydropsychids (Table 7.3). This indicates that hydropsychids remove dinoflagellates and certain groups of diatoms in lower proportions than their presence in the seston. In the summer, the seston had a higher proportion of one green algal marker, again indicating a difference in its uptake by hydropsychids (Table 7.3). Overall, hydropsychids had significantly higher levels of many of the markers compared to the seston, including diatoms (20:5ω3), green algae (18:3ω3), golden brown algae, cyanobacteria, terrestrial material, carnivory (although both markers were only higher in the summer), all essential fatty acids and essential HUFA fatty acids (Table 7.3). These results show that hydropsychids were using food in proportions different from those in the seston. In general, therefore, hydropsychids were selectively utilizing the seston as a food source.

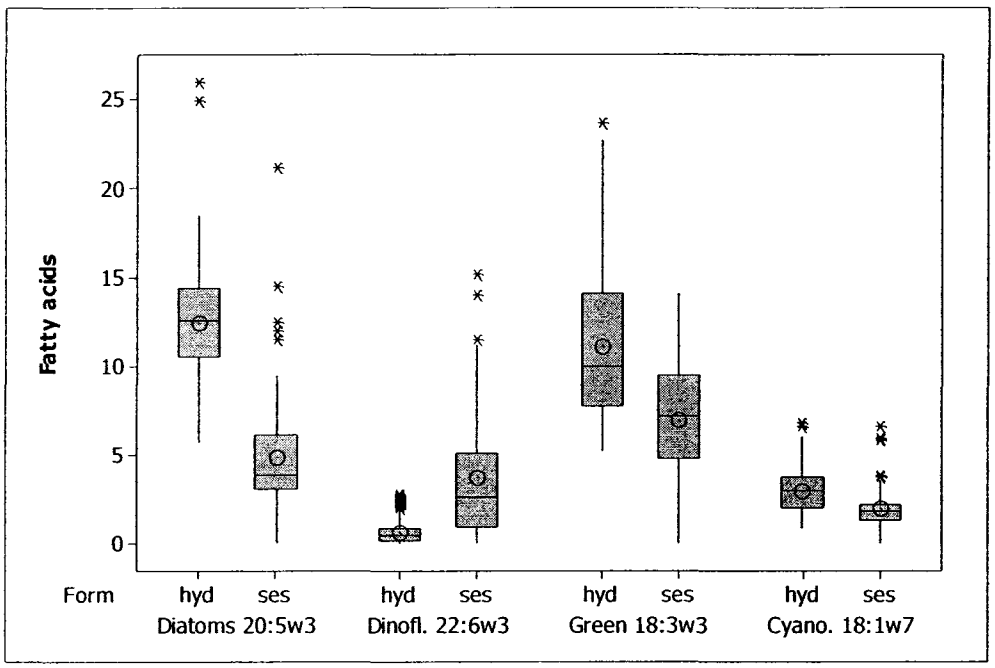

Figure 7.6 Boxplots of fatty acids by group, using both seasons where hyd = hydropsychids and ses = seston. Asterisks depict outliers, boxes show the interquartile range, and the dotted circles depict the mean. Dinofl. = dinoflagellates, Cyano = cyanobacteria.

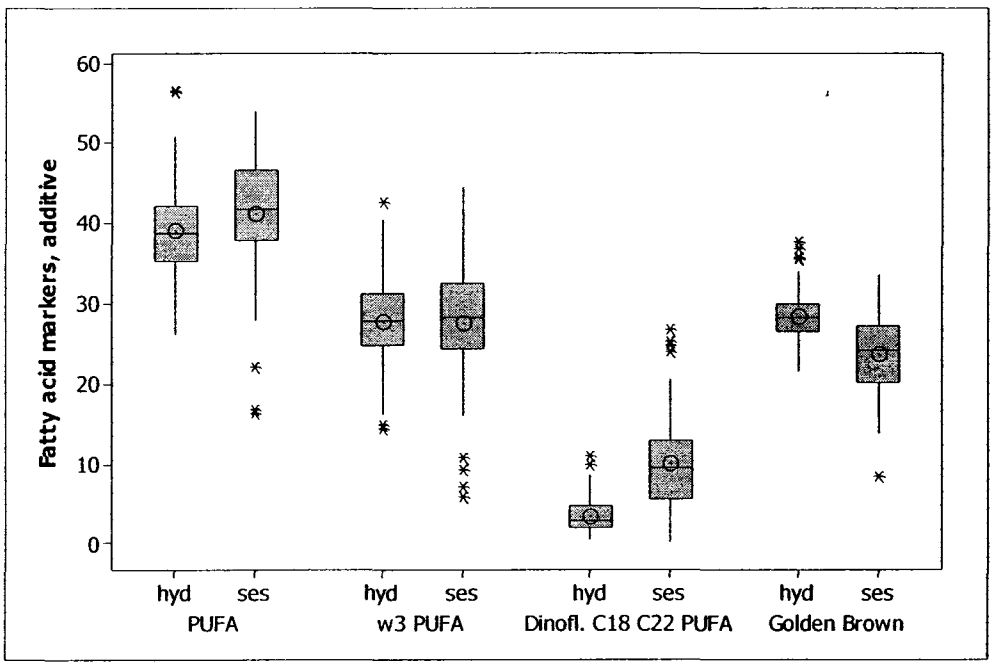

Figure 7.7 Boxplots of fatty acid markers by group, using both seasons where hyd = hydropsychids and ses = seston. Asterisks depict outliers, boxes show the interquartile range, and the dotted circles depict the mean. Dinofl. = dinoflagellates.

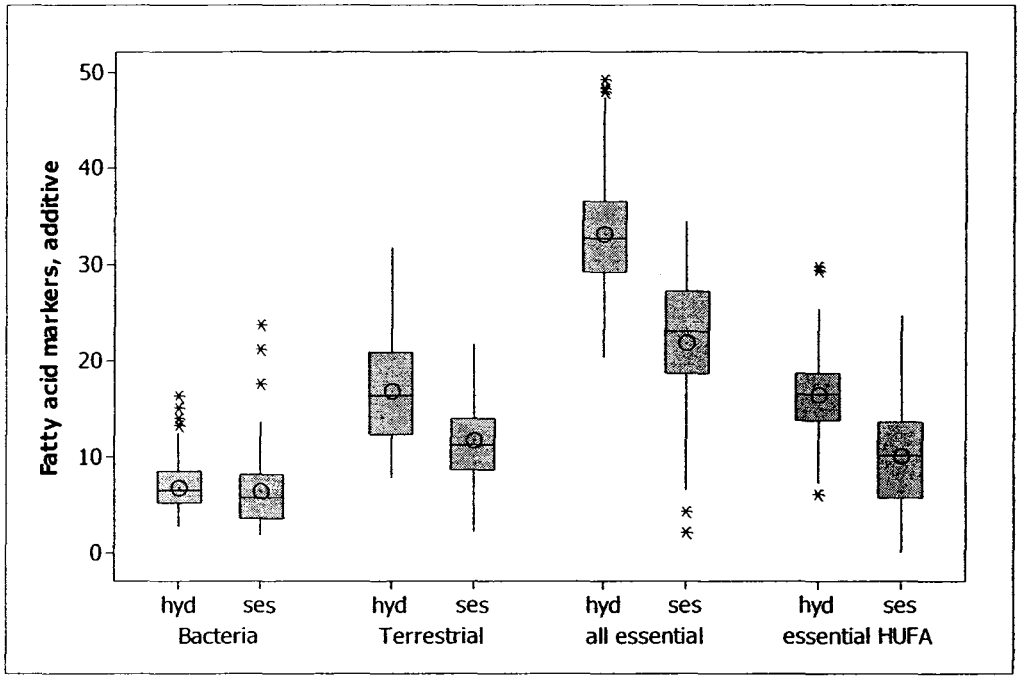

Figure 7.8 Boxplots of fatty acid markers by group, using both seasons where hyd = hydropsychids and ses = seston. Asterisks depict outliers, boxes show the interquartile range, and the dotted circles depict the mean..

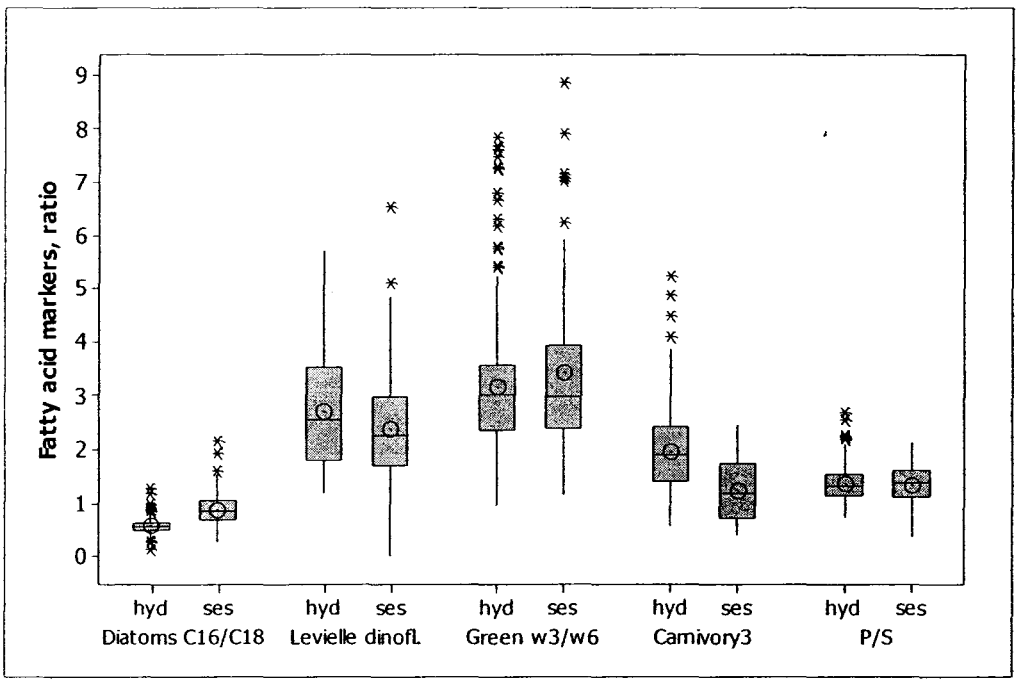

Figure 7.9 Boxplots of fatty acid markers by group, using both seasons where hyd = hydropsychids and ses = seston. Asterisks depict outliers, boxes show the interquartile range, and the dotted circles depict the mean. Dinofl. = dinoflagellates.

Table 7.3 Table of p values for fatty acid markers, comparing differences among seston and hydropsychids with season. Bolded items differ with season.

Fatty acid marker		spring only		summer only		both seasons	
		p value	higher	p value	higher	p value	higher
General algae	PUFA	ns		ns		**0.016**	**ses**
	sum ω3	ns		ns		ns	
Diatoms	20:5ω3	<0.0001	hyd	<0.0001	hyd	<0.0001	hyd
	$\Sigma C_{16}/\Sigma C_{18}$	**ns**		<0.0001	ses	<0.0001	ses
	Leveille diatoms	<0.0001	ses	<0.0001	ses	0.002	ses
Dinoflagellates	22:6ω3	<0.0001	ses	<0.0001	ses	<0.0001	ses
	$\Sigma C_{18} + \Sigma C_{22}$ PUFA	<0.0001	ses	<0.0001	ses	<0.0001	ses
Green	18:3ω3	<0.0001	hyd	<0.0001	hyd	<0.0001	hyd
	ω3/ω6	ns		**0.002**	**ses**	ns	
Golden brown	16:0+18:1ω9	<0.0001	hyd	<0.0001	hyd	<0.0001	hyd
Cyanobacteria	18:1ω7	0.005	hyd	<0.0001	hyd	<0.0001	hyd
Bacteria	sum (see text)	**<0.0001**	**ses**	**0.003**	**hyd**	**ns**	
Terrestrial	18:3ω3+18:2ω6	<0.0001	hyd	<0.0001	hyd	<0.0001	hyd
Carnivory	18:1ω9/(16:1ω7+18:1ω7)	<0.0001	hyd	<0.0001	hyd	<0.0001	hyd
	PUFA/SAFA (P/S)	ns		**0.027**	**hyd**	ns	
all essential	sum (see text)	<0.0001	hyd	<0.0001	hyd	<0.0001	hyd
essential hufa	sum (see text)	<0.0001	hyd	<0.0001	hyd	<0.0001	hyd

7.4 Discussion

7.4.1 Lipid classes: comparison of seston and Hydropsychidae

The lipid class composition of the seston, despite its variability, clearly differed from that of hydropsychids (Figure 7.1 & Figure 7.2). Hydropsychids had a high proportion of TAG, presumably because digested seston is converted primarily into this storage lipid (Parrish et al. 2000). This reflects the need for hydropsychid larvae to store energy for use during pupation, adulthood and reproduction because pupae and adults do not feed (Wiggins 1996).

To better elucidate processes that result in differences in lipid class composition between seston and hydropsychids a better understanding of food selectivity by hydropsychids, as well as the digestion and assimilation rates of food ingested, is needed.

Previous research on the phytoplankton assemblage of Newfoundland lakes indicated an average of 46 taxa per lake (Scruton et al. 1987), and so the phytoplankton drifting from the lentic systems will be quite varied. There is also a diversity of zooplankton in Newfoundland lakes. Chengalath et al. (1984) recorded 68 species of rotifers and crustaceans and Knoechel & Campbell (1988) reported that the biomass of Newfoundland zooplankton, including cladocerans, copepods and rotifers, was similar to lakes elsewhere. This illustrates the potential diversity of the food items available to hydropsychid larvae especially at lake outlets.

Seston was more variable on PCA2 than hydropsychids, because of the stochastic nature of the seston within and among streams (Figure 7.2). This was evident by the high degree of variability amongst the three replicates collected at each site; since samples were collected in under one hour, this indicates how patchy seston can be over a short time period. To better characterize the seston composition and quality that is available to hydropsychid larvae, more extensive research on stream seston dynamics is needed to better define the variability of its composition in space and time as well as its quality as a hydropsychid food source. Larval lipid composition is an integration of some portion of the variable potential diet available through time.

7.4.2 Fatty acids: comparison of seston and Hydropsychidae

The fatty acid composition also differed between hydropsychids and seston. This was most distinct when all 65 fatty acids were used in a PCA analysis where hydropsychids scored low on PCA1 which corresponded with 20:5ω3 (Figure 7.3). This is eicosapentaenoic acid (EPA), a general diatom marker and an essential fatty acid. It is

unclear whether the difference is caused by food sources or *de novo* synthesis, as capabilities for synthesis by hydropsychids are unknown. This result is supported by Persson & Vrede (2006)who reported higher proportions of 20:5ω3 in zooplankton compared to seston in oligotrophic Swedish lakes. Hessen & Leu (2006) also found higher proportions of 20:5ω3 for *Daphnia* compared to seston in high Arctic lakes despite great variation between lakes.

Longer chain saturated fatty acids are generally indicative of terrestrial material (Dalsgaard et al. 2003). In this study 23:0, 24:0 were associated with seston, but were not associated with the hydropsychids. This suggests that hydropsychids do not select terrestrial material from the seston.

Spring samples of both hydropsychids and seston scored higher on PCA2 (Figure 7.3) which was positively correlated with 15:0*i*, 16:0*i* and 16:0*ai*. These are bacterial markers indicating there may be more bacteria present in the spring. PCA2 negatively correlated with 22:0 and 22:1ω11(13) which are general zooplankton markers indicating there may be more zooplankton in the summer (Dalsgaard et al. 2003).

The PCA using the 14 dominant fatty acids did not show strong segregation between the seston and the hydropsychids, nor was there a strong correlation with a group of fatty acids. This indicated that there was a general similarity in the composition of these 14 dominant fatty acids between the seston and hydropsychids, i.e. both contained similar fatty acids but in different proportions and thus the fatty acid composition of the seston is reflected in the hydropsychids. The hydropsychids tended to be dominated by a few fatty acids whereas seston had lower proportions of several fatty

acids, including those that were dominant in the hydropsychids. Using only the 14 dominant fatty acids, both seston and hydropsychids demonstrated a similar seasonal shift (Figure 7.4), suggesting that the hydropsychid fatty acid composition was responding to the available food resources and thus that hydropsychids were opportunistically feeding. The results of the current study indicate that fatty acid analysis can provide information on food resource utilization by hydropsychids, reinforcing the value of gaining a better understanding of fatty acids in the seston.

Spring seston samples were from 3 L of water filtered in the lab, while summer samples were from 50 L of water sieved in the field using a 10 μm mesh. As fatty acid composition was expressed as a percentage of identifiable fatty acids, comparisons between spring and summer were technically possible based on the relative proportions. Sieving samples in the field would underestimate the contribution of organisms smaller than 10 μm, such as bacteria. Consequently, the finding that spring seston samples showed higher proportions of bacterial markers compared to the summer seston samples needs further research. However, the summer samples did contain a greater volume of material and so provided a better characterization of the stream seston.

Fatty acid markers provided some insight into seston composition. However, because these markers were not derived from lotic systems their applicability is limited. Nevertheless, the fatty acid markers highlighted that there were differences between the fatty acid composition of the seston and that of the hydropsychids. The results provided evidence that hydropsychids were utilizing food in proportions different from those in the seston, suggesting selective ingestion. One method of seston selection could be caused by

hydropsychid nets clogging and/or flow dynamics, which would differentially trap a certain portion of the seston (Fuller et al. 1983; Fuller & MacKay 1980a). Hydropsychids are also known to selectively consume food resources off their nets (Fuller & Mackay 1981; Petersen 1985; Petersen 1987a). This selective process is well known in insects in general (Nation 2002). Hydropsychids had higher proportions of multiple fatty acid markers, indicative of several groups of organisms, compared to the seston which suggests that hydropsychids were highly omnivorous, utilizing a wide variety of food resources as previously reported using other techniques (Alstad 1987; Merritt & Cummins 1996). Therefore the data from this study indicate that Newfoundland hydropsychids are opportunistic feeders, capable of responding to changes in resources among streams and along seasonal gradients.

7.5 Conclusion

Hydropsychids and seston clearly differed in their lipid class compositions, with hydropsychids having a higher proportion of TAG than seston samples. As TAG is a storage lipid, it might be expected to dominate hydropsychids given that pupae and adults do not feed. Fatty acid composition also clearly differed between seston and hydropsychids. However, this could not be attributed to a given group of fatty acids. Hydropsychids obtained their food from the seston and thus much overlap between the two groups might be expected if hydropsychids were not selective in terms of what they ingested and/or digested. Hydropsychid lipids and fatty acids were much less variable than those of the seston, demonstrating the stochastic nature of the stream seston. However, hydropsychid lipid composition represents dietary intake over time, but the

seston lipid composition was only a brief snapshot in time. It is evident that much more research is needed to determine patterns of seston composition, quantity and quality at both the spatial and temporal scale.

Fatty acid markers showed many differences between hydropsychids and seston, indicating both selectivity and opportunism in the feeding of the hydropsychids. The data indicate a high degree of omnivory by all the hydropsychid species here regardless of location in the stream or net mesh-size. Hydropsychids removed higher proportions of the Carnivory3 marker, and the P/S marker in the summer, indicating the importance of protein sources in their diet. They also had higher proportions of the essential fatty acids indicating that they were able to either selectively remove/retain this material and/or produce it *de novo*. This study indicated selective feeding differences by hydropsychids, but also demonstrated an ability to adapt to differing food sources both among and within streams, showing that the depauperate Newfoundland hydropsychid fauna is composed of opportunistic generalists which can adapt to a wide diversity of stream habitats.

8. CHAPTER 8: SUMMARY AND FUTURE RESEARCH DIRECTIONS

Species of the impoverished Newfoundland Hydropsychidae fauna exhibited niche breadths similar to those occupied on the mainland where these species co-exist with a much greater diversity of Hydropsychidae as well as increased numbers of other groups. The broad survey, which incorporated presence/absence data from multiple studies, showed that species were distributed across the island and did not show regional differences (Chapter 2). No new species were recorded for the island. *Cheumatopsyche pettiti* was narrowly restricted to outlets in Newfoundland which were generally warmer and had increased food resources. *Hydropsyche betteni* and *H. alternans* were also mostly restricted to outlets, but often their distribution extended much further downstream than that of *C. pettiti*. *Hydropsyche alternans* had a much lower frequency of occurrence than the two other outlet species. *Hydropsyche slossonae* and *Hydropsyche sparna* were generalists in that they were able to occur throughout the length of streams. Primarily downstream species, which occurred less frequently, were *A. ladogensis,* found mainly in larger streams and *D. modesta,* found only in few downstream localities. Although *D. modesta* was recorded elsewhere on the island (Marshall & Larson 1982) it was the most restricted species studied. *Parapsyche apicalis* was widespread but restricted to cooler, generally small streams. In general forested streams had higher densities of larvae, were warmer and had more food resources compared to barren streams.

The challenge of comparing forested and barren streams over a wide range of sizes was addressed by deriving a model which used a negative exponential function with a slope of -0.5878. This function related width at a riffle to discharge and thus the

potential energy available to maintain suspended particles. Using this function, five sampling stations at relative distances from outlets were established in eight streams ranging from zero to 23 metres wide. This approach allowed investigation into the rate of change of hydropsychid abundance with increasing distance from outlets (longitudinal distribution). Species abundances did show similar trends across all eight streams suggesting that the derived exponential function was modeling resource changes and the natural change in abundances of the hydropsychids. Species commonly occurring at outlets (*C. pettiti, H. betteni, H. alternans*) had rapid declines in abundance with distance from outlets in all streams where they occurred. Species that frequently occurred in higher abundances downstream than at outlets (*H. slossonae, H. sparna, A. ladogensis, D. modesta*) showed either no longitudinal change in abundance or had increased abundances downstream, indicating that they were responding to factors other than those generated by the presence of a lake outlet. The abundance of potential food resources from an outlet, lake phytoplankton and zooplankton, also followed a negative power function below outlets, exhibiting a rapid decline in abundance downstream. Periphyton abundances showed no marked difference among these eight streams, nor did its longitudinal abundance change greatly indicating that conditions for its growth remained fairly stable throughout streams.

The pattern of periphyton abundance along streams showed a similar distribution to that of *H. slossonae* and *H. sparna*. Phytoplankton and zooplankton abundances and those of certain hydropsychid species showed similar trends in individual streams (section 4.3.3). The rate of decline in *H. betteni* abundance using all eight streams more

consistently followed that of zooplankton. This trend also held true for total hydropsychids in barren landscapes. Thus, this study did find similar trends between abundances of hydropsychids and their potential food resources. However, this did not indicate what elements of this potential food source were being utilized, nor was the influence of location and landscape known using this coarse scale of food quantity. Another question unanswered by the exponential model was whether these food resources were being partitioned among the hydropsychid species.

Lipid and fatty acid analyses allowed a more detailed examination of the feeding ecology of Newfoundland hydropsychids. Previous studies had investigated the fatty acid composition of only two hydropsychid genera, but not in terms of location or landscape. The current study was the first known analysis to consider lipid and fatty acid composition among closely related riverine organisms.

Lipid analyses showed hydropsychids to be mostly composed of TAG, a storage lipid, indicating their diets were sufficient to accumulate fat. Comparing TAG proportions by species, life stage, season, stream, location or landscape, only outlets versus downstream differed significantly. This difference was only pronounced in the spring samples. This indicated that outlets provided higher quality and/or quantities of food sources.

Fatty acid composition of the species was very similar with the same 14 of the 65 identified fatty acids being above 1% in each species. *Parapsyche apicalis* had the most distinctive composition, followed by *D. modesta, A. ladogensis* and *H. alternans*. The four most commonly occurring species had very similar fatty acid compositions

indicating that they were utilizing similar foods and/or had similar assimilation capacities. Similarities could also result from lipid metabolism, where fats may be converted to similar fatty acids for storage in fat bodies in all these closely related species. Likewise the differences between *P. apicalis* and *D. modesta* could arise from different metabolic pathways because they do differ taxonomically from the other species. Persson & Vrede (2006) found taxon-specific differences in lentic zooplankton fatty acid composition to be partly attributable to phylogenetic origin, thus this area requires further exploration.

Little was known of the lipid content of stream seston, so the spring samples were preliminary and provided an estimate of the volumes of material required for analyses. There was considerable variance in the lipid and fatty acid composition of the seston among streams. Some phytoplankton species are known to be more abundant at outlets than downstream, as are the zooplankton taxa, and these food resources may also have been influenced by nutrients and temperature which would have increased their variability.

The fatty acids of hydropsychids did show different proportions compared to their abundance in the seston, indicating hydropsychids were selectively ingesting and/or digesting certain foods. There was also a similar seasonal shift, indicating that hydropsychids were able to adapt to changing food resources. Individual species did not show clear differentiation among streams, locations or landscapes, indicating that they were opportunistically utilizing suitable food sources at a site. Lack of strong

differentiation among species indicated that they were not partitioning their food sources. Thus Newfoundland Hydropsychidae were opportunistic generalists.

The current study has shown that the Newfoundland hydropsychid species, which are widely distributed in North America, occupy similar niches in Newfoundland with its depauperate fauna as they do in areas with high numbers of species. In fact, they were more restricted here, with *C. pettiti* only found at lake outlets and *D. modesta* limited to narrow sections of only a few streams. As both forested and barren streams were colonized by all the species, this factor was not critical for habitat selection. Also demonstrated was that all species had very broad and very similar dietary intakes as indicated by the comparison of lipid composition among species and by the comparison of hydropsychids and their potential food resources. This leads to a conclusion of all species being opportunistic feeders and omnivorous to varying degrees. This has implications for attempts to allocate organisms to levels in food webs and for tracking trophic relationships through lotic systems. Stable isotope analysis combined with the use of fatty acid markers is one applicable method for future research, a technique which has been used to track trophic relationships in lentic zooplankton (Perga et al. 2006).

This raises several critical questions for future research that would make major contributions to our ecological understanding of hydropsychids, both on broad and restricted scales. As illustrated by the patchy distribution of *H. alternans* and *D. modesta* on the island, we have a poor understanding of the cues species use to select habitat. A better understanding of adult behaviour, cues used to select mating and oviposition sites and distances adults can fly are critical to any environmental management and

conservation plan. Determining the quality of a habitat from an organism's perspective is also vital. Larval transplant experiments using artificial channels situated along different stream habitats to examine growth patterns when larvae are exposed to different streams and stream sections (i.e. outlets, downstream, small and large streams) would be very informative. This would provide an understanding of the influence of food resources and allow for comparative analyses on the same hydropsychid population exposed to different potential foods. This approach could be designed to provide critical experimental information on hydropsychid life cycles and the affects of inter- and intra- species competition, as well as the impacts of other non-hydropsychid species on their distribution and development.

This raises the question of understanding the interaction of a filter feeding guild in the field, particularly where they occur in high densities at outlets. This would include species composition and life history, and ingestion, assimilation and egestion rates of size-class ranges of seston. Rates and size ranges of seston utilized would depend on the hydropsychid life cycle. Tracer dyes, as used by Wotton et al. (1995), would be valuable as would comparative lipid analyses of co-occurring members of the guild. These could then be tested in field trials across a range of discharge rates because these are related to filter feeder abundance (Vadeboncoeur 1994). Another aspect is the importance of spates that cause flushing of seston from lakes carrying it greater distances downstream (Campbell 2002). This improved knowledge of seston dynamics in relation to filter feeders would improve modeling of filter feeder abundance and that of the stream benthic community in general.

Research is required on lipid class and fatty acid composition of freshwater organisms, particularly in lotic systems. This would verify the applicability of using one and/or several fatty acids as a marker that is indicative of freshwater organism(s) which could be used to determine if consumers, such as hydropsychid larvae, are ingesting a particular food type. This could be done by comparative laboratory feeding experiments of seston and algal cultures as conducted by Acharya et al. (2005), which helped determine the nutritive value of the seston. Feeding experiments would also determine ingestion, assimilation and digestion rates of lipids and the dynamics of fatty acid transfer, assimilation and storage through the food web. This would also allow investigation into capabilities and rates of synthesis of fatty acids *de novo*. Digestive processes involving lipids are not well known, nor is the metabolism of stored fats. Deficiencies of PUFA, particularly linoleic and linolenic acids, have been shown to hamper ecdysis and impair adult morphology and fecundity in lepidopterans, hymenopterans and coleopterans (Nation 2002). Dipterans are unable to biosynthesize PUFAs, although a few insects such as a cricket (*Acheta domesticus*) and a cockroach (*Periplaneta americana*) are able to synthesize PUFAs (Nation 2002). Fast (1964; 1970) reviewed insect lipids in about 35 species and this still accounts for most of our studies on lipid physiology today (Klowden 2002).

Lipid analysis has useful possibilities in ecological research for investigating dietary intake over time, and following the diet of a cohort would detect ontogenic shifts as suspected in hydropsychids. Continuous sampling would explore seasonal dynamics which would better characterize a stream and/or organism. As lipid analysis allows large

sample sizes, multiple streams and/or organisms could be considered simultaneously to compare dynamics within and among systems and help to understand how habitat influences the dietary needs of consumers. This could then be expanded to larger spatial scales or a guild of organisms or could follow trophic transfers. Such studies are important for understanding the broader dynamics of lotic systems.

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10. APPENDICES

10.1 Appendix 1: North American Distribution of Newfoundland Hydropsychidae (after Nimmo (1987))

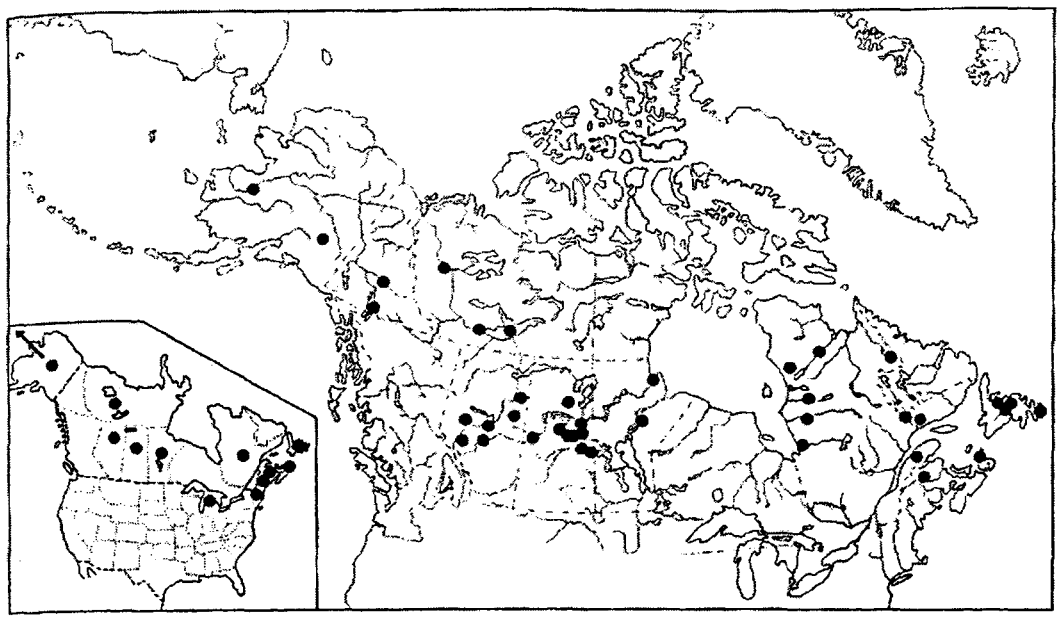


Figure 10.1 North American distribution of *Arctopsyche ladogensis*. Large map: Canada and Alaska. Inset map: general North America.

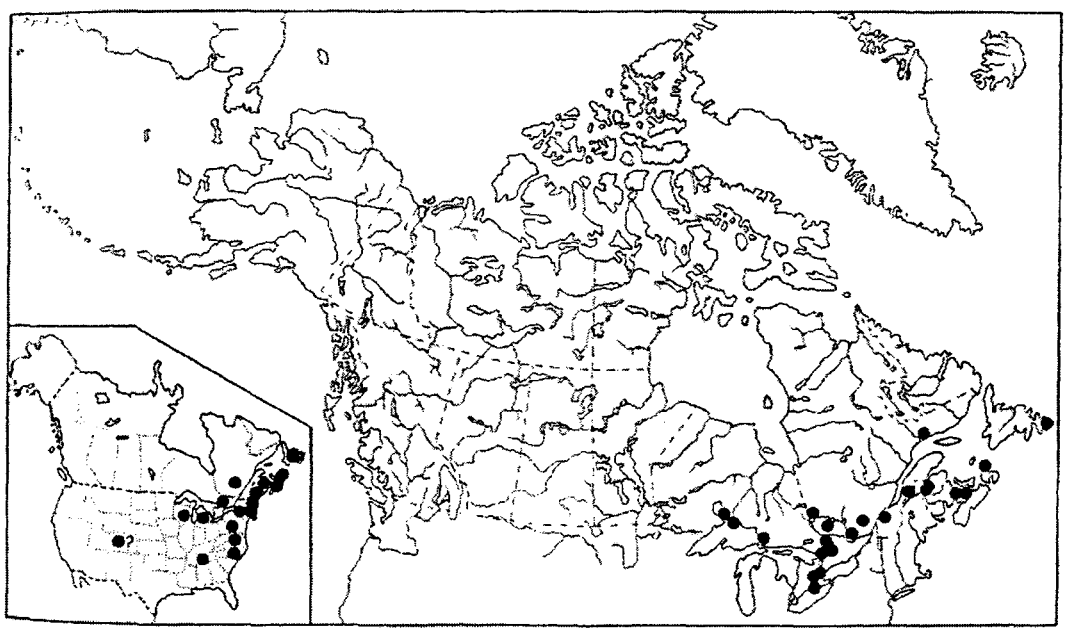


Figure 10.2 North American distribution of *Parapsyche apicalis*. Large map: Canada and Alaska. Inset map: general North America.

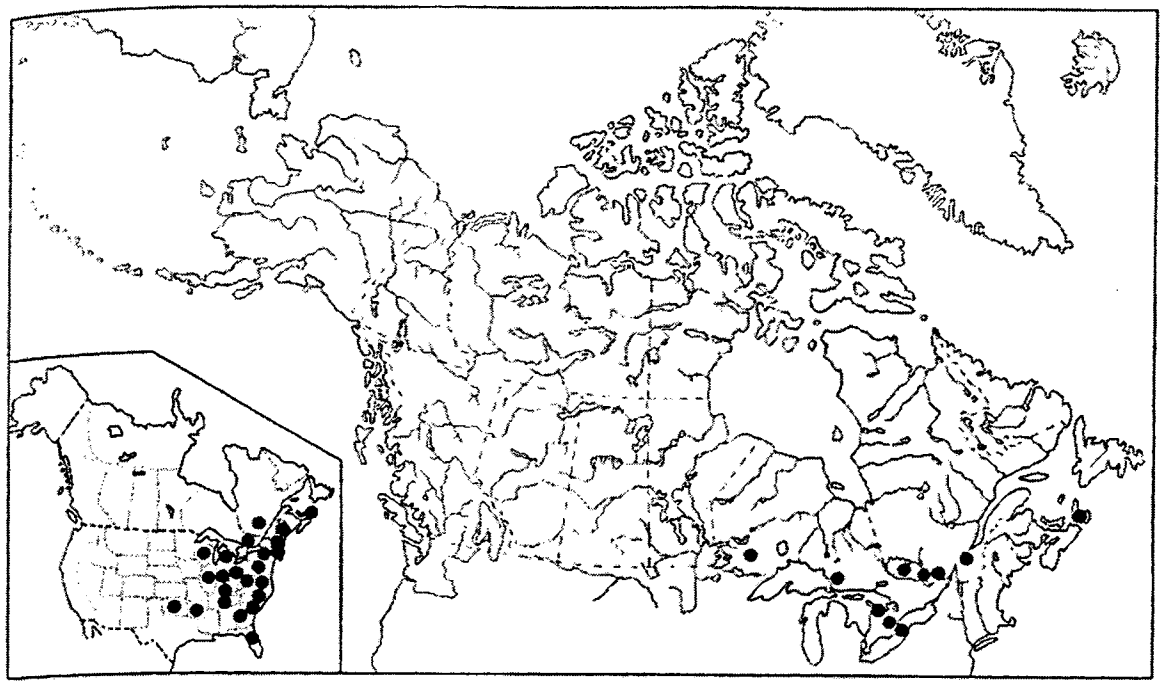


Figure 10.3 North American distribution of *Diplectrona modesta*. Large map: Canada and Alaska. Inset map: general North America. Recorded in Newfoundland by Marshall & Larson (1982).

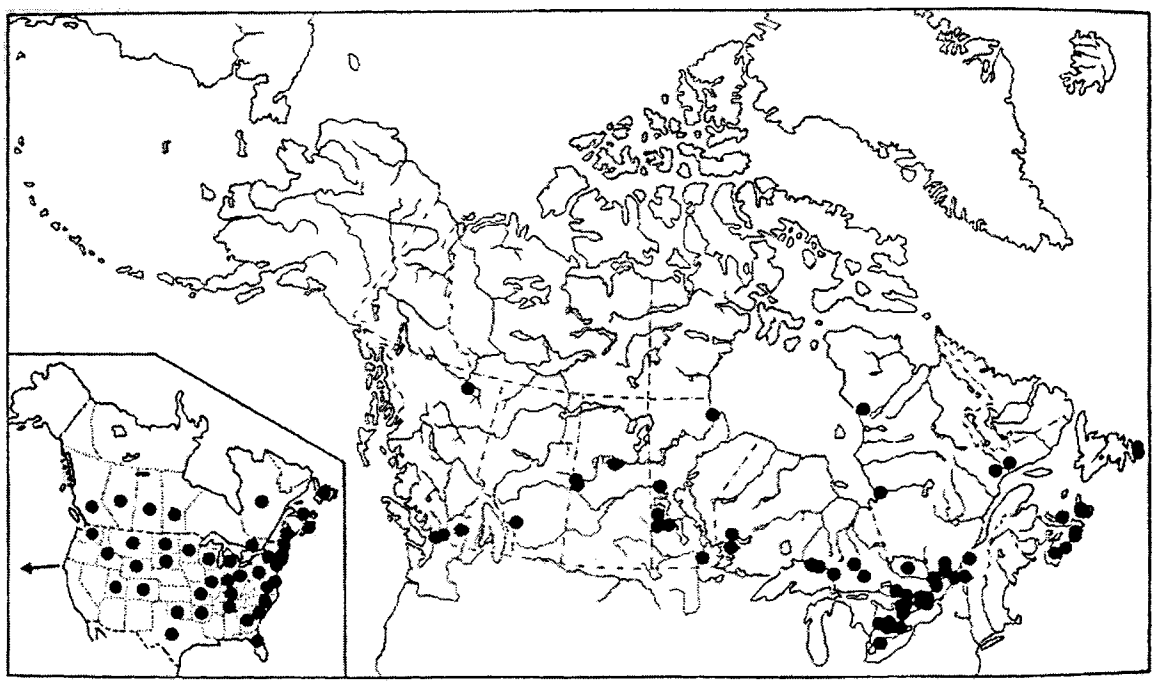


Figure 10.4 North American distribution of *Cheumatopsyche pettiti*. Large map: Canada and Alaska. Inset map: general North America.

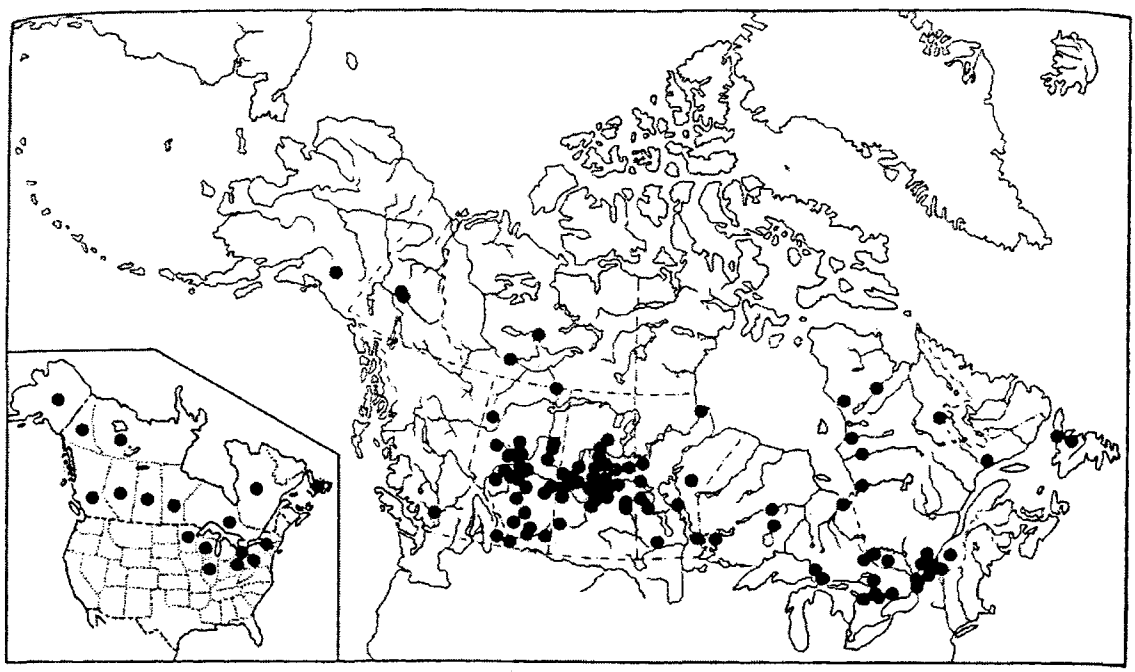


Figure 10.5 North American distribution of *Hydropsyche alternans*. Large map: Canada and Alaska. Inset map: general North America.

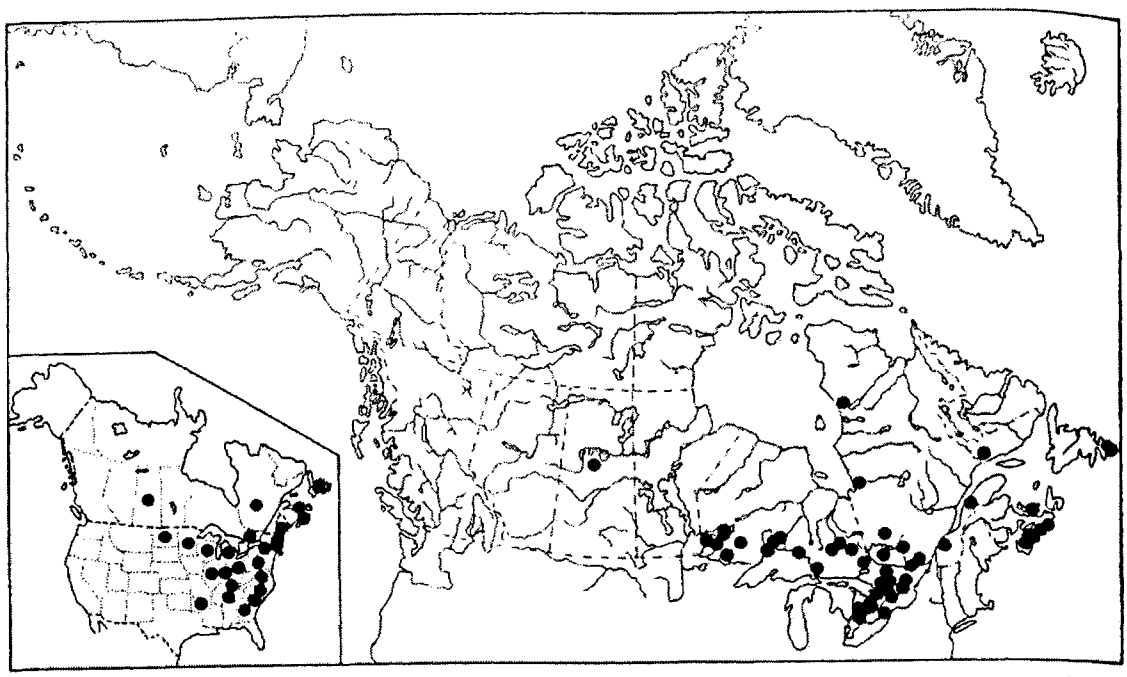


Figure 10.6 North American distribution of *Hydropsyche betteni*. Large map: Canada and Alaska. Inset map: general North America.

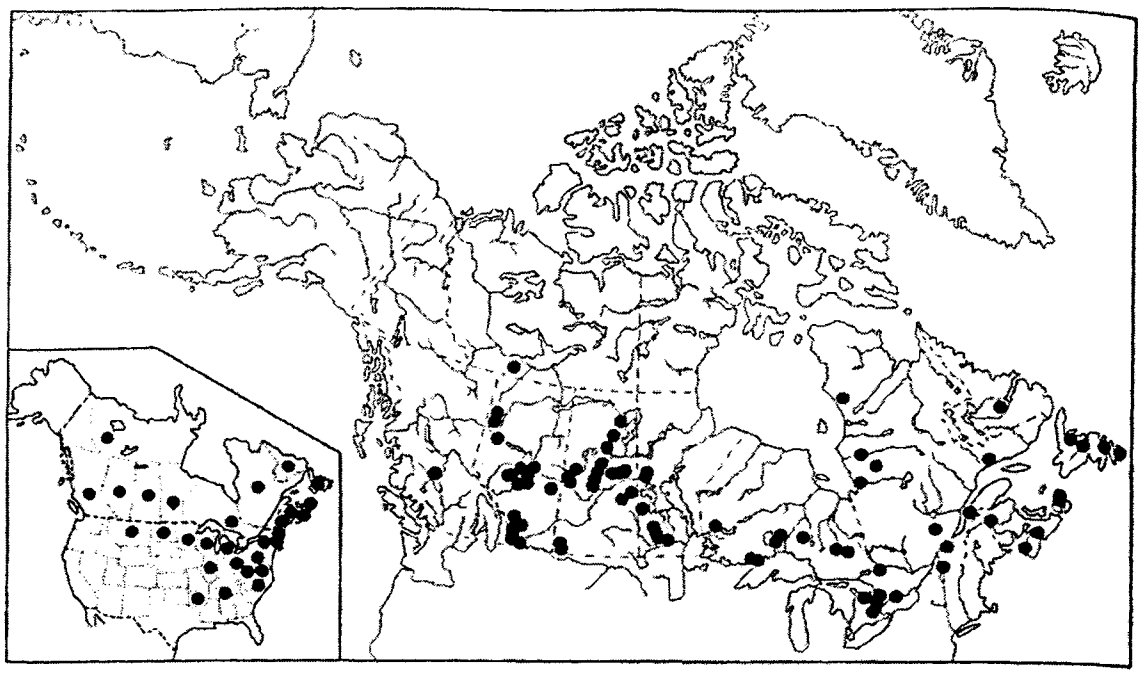


Figure 10.7 North American distribution of *Hydropsyche slossonae*. Large map: Canada and Alaska. Inset map: general North America.

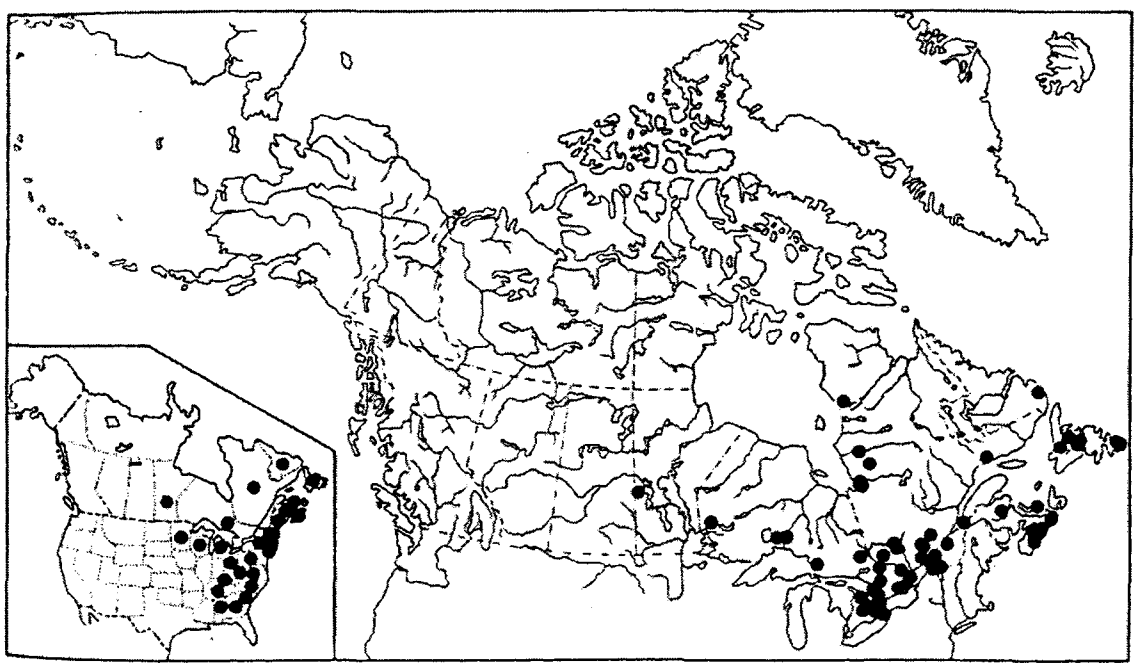


Figure 10.8 North American distribution of *Hydropsyche sparna*. Large map: Canada and Alaska. Inset map: general North America.

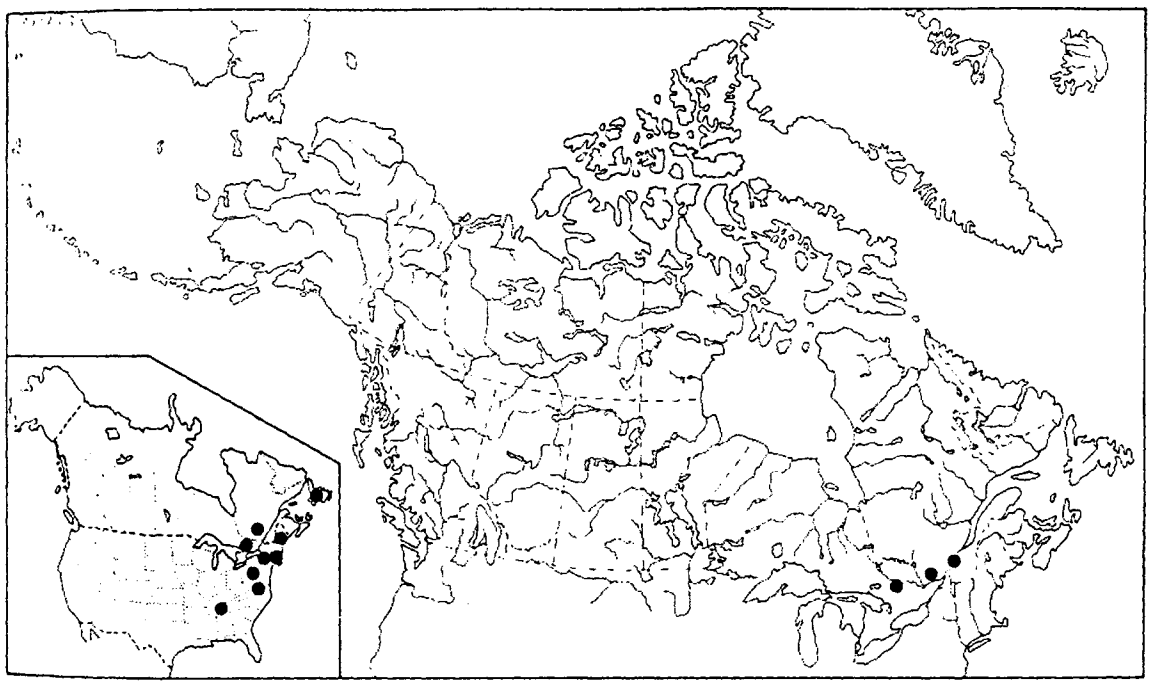


Figure 10.9 North American distribution of *Hydropsyche ventura*. Large map: Canada and Alaska. Inset map: general North America.
Note: this species is marked as occurring in Newfoundland on the inset map but this data point is missing on the large map.

10.2 Appendix 2: Pictures of selected study sites

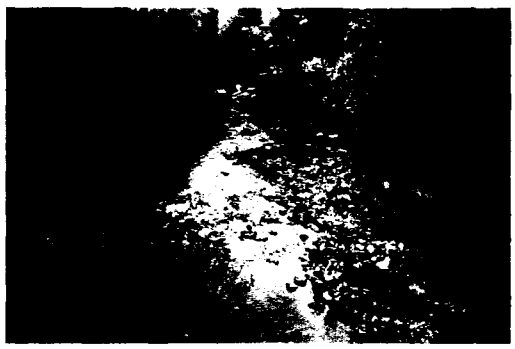
Fitzgerald outlet, September 14 2001

Rocky Harbour River, September 24 2001

Fitzgerald downstream (NorthEast River), Sept. 14 2001

Colinet River, September 24 2001

Monolith outlet, September 16 2001



Broad Cove downstream, October 24 2001

Monolith downstream, September 16 2001

Cape Race, May 2 2002

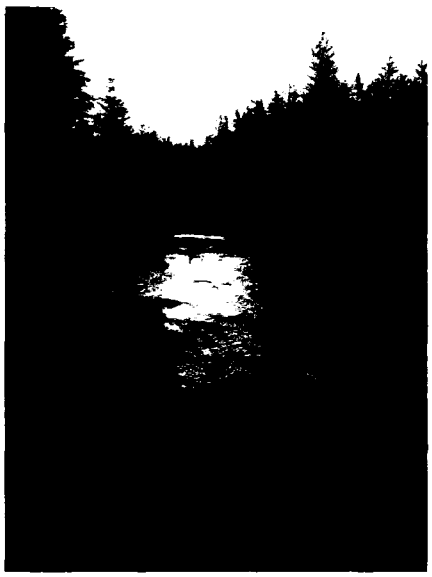
Figure 10.10 Photographs of some of the 96 field sites sampled with a surber for Chapter 2.

Broad Cove outlet, facing upstream, August 2006

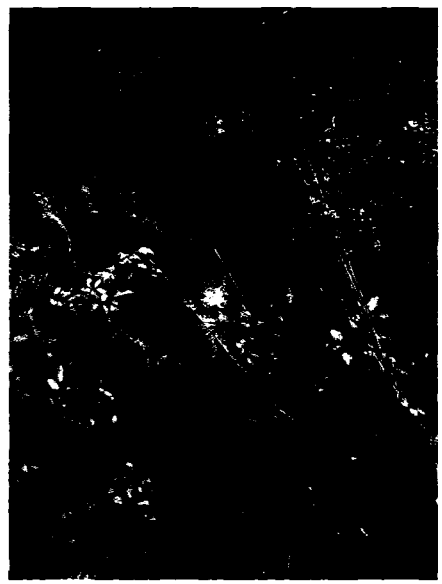
Barking Kettle outlet, August 2006

Broad Cove outlet, sampling site, August.2006

Barking Kettle downstream, August 2006



Broad Cove downstream, August 2006



Barking Ketlle downstream, wide angle, August 2006

Figure 10.11 Photographs of outlets and downstream sites of Barking Kettle and Broad Cove, surber sampled for the broad survey of Chapter 2, sampled with rock bags for Chapter 4 and collection sites for lipid analyses of Chapters 5-7.

Great Pond outlet, August 26 2004

Beaver Pond outlet, August 25 2004

Great Pond downstream, August 26 2004

Beaver Pond downstream, August 25 2004

Figure 10.12 Photographs of outlets and downstream sites of Great Pond and Beaver Pond, surber sampled for the broad survey of Chapter 2, sampled with rock bags for Chapter 4 and collection sites for lipid analyses of Chapters 5-7.

Split Rock outlet, May 23 2004

Walking into Above Hatchet, June 1 2004

Split Rock downstream, May 23 2004

Above Hatchet outlet, June 1 2004

Split Rock downstream, August 22 2004

Near Above Hatchet outlet, August 27 2004

Above Hatchet downstream, Aug 27 2004

Figure 10.13 Photographs of outlets and downstream sites of Split Rock and Above Hatchet sampled for the broad survey of Chapter 2, sampled with rock bags for Chapter 4 and collection sites for lipid analyses of Chapters 5-7.

Watern outlet, May 15 2003

Watern downstream, August 30 2004

Watern outlet, June 1 2004

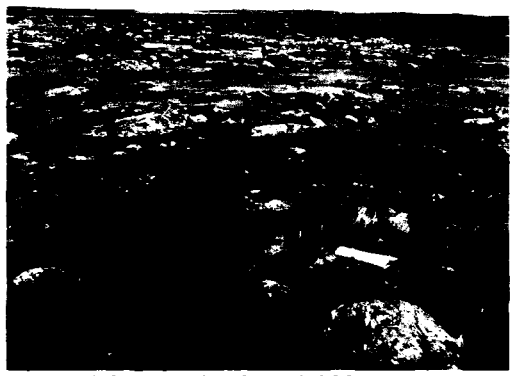
Walking into Watern, May 15 2003

Watern downstream, May 15 2003

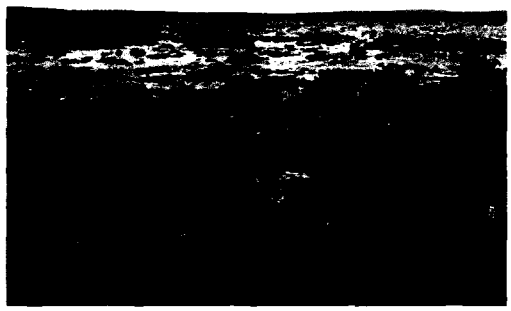
View of lake on Watern, May 15 2003

Rock bag in Watern, May 15 2003

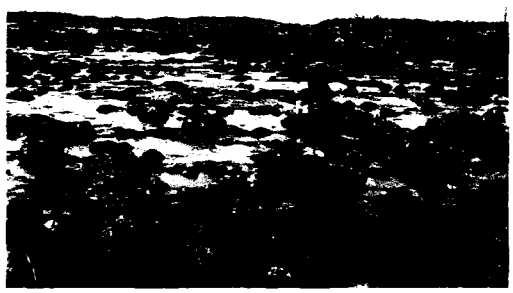
Figure 10.14 Photographs of Watern, rock bag sampling was conducted in 2003 and lipid sampling was conducted in 2004.



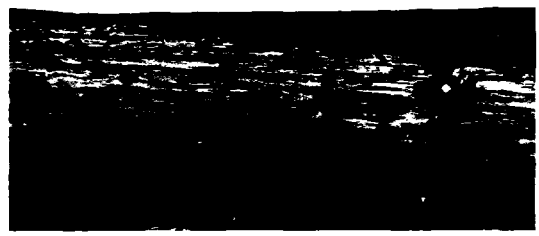
Portugal Cove outlet, June 3 2004



Portugal Cove outlet, August 29 2004

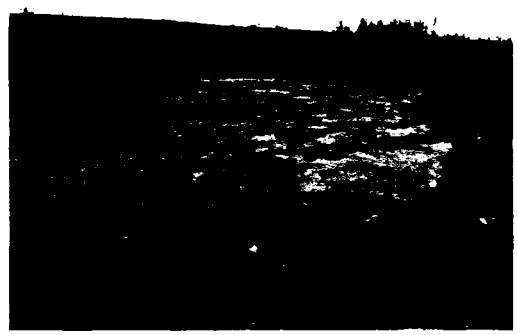


Portugal Cove Station 2, May 15 2003



Portugal Cove Station 4, May 15 2003

Portugal Cove Station 6, May 15 2003



Portugal Cove Station 8, May 15 2003

Rock bag in Portugal Cove, May 15 2003

Figure 10.15 Photographs of Portugal Cove, rock bag sampling was conducted in 2003 and lipid sampling was conducted in 2004. Surber samples were also collected in 2001.

10.3 Appendix 3: A brief introduction to lipids and fatty acids

Lipids are important for energy storage, for metabolic requirements, as components of hormones, and are essential to the structure of cell membranes and cuticles (Downer 1978). Of the aquatic insects studied, most had a total lipid content of 10-20% of total insect dry weight (Hanson et al. 1985).

Lipids are a heterogeneous group of compounds with two properties: 1) relatively insoluble in water and 2) soluble in nonpolar solvents such as chloroform (Downer 1978). A lipid extract may contain as many as 16 subclasses of lipids (Parrish 1999). Two lipid classes, phospholipids and sterols (shown in Figure 5.1), are essential to the structure of cell membranes (Parrish 1988). The main class of lipids in most organisms is triacylglycerols (shown in Figure 10.16 & Figure 10.17), composed of glycerol and fatty acids. These can be used for energy storage, buoyancy control or thermal insulation (Bell et al. 1994; Parrish 1988). Triacylglycerols (TAG) are a large component of the lipid (at least 35% of total lipids) of freshwater macroinvertebrates (Bell et al. 1994) and are necessary for metamorphosis and reproduction (Cargill et al. 1985).

Most biogenic lipid classes occur as esterified acyl lipids, where an acyl group is part of the molecule linking fatty acids to a glycerol backbone (Figure 10.17 & Figure 10.18) (Parrish 1999). The fatty acids can be cleaved from this backbone, re-esterified to methyl esters, and then analyzed by gas chromatography (Parrish 1999).

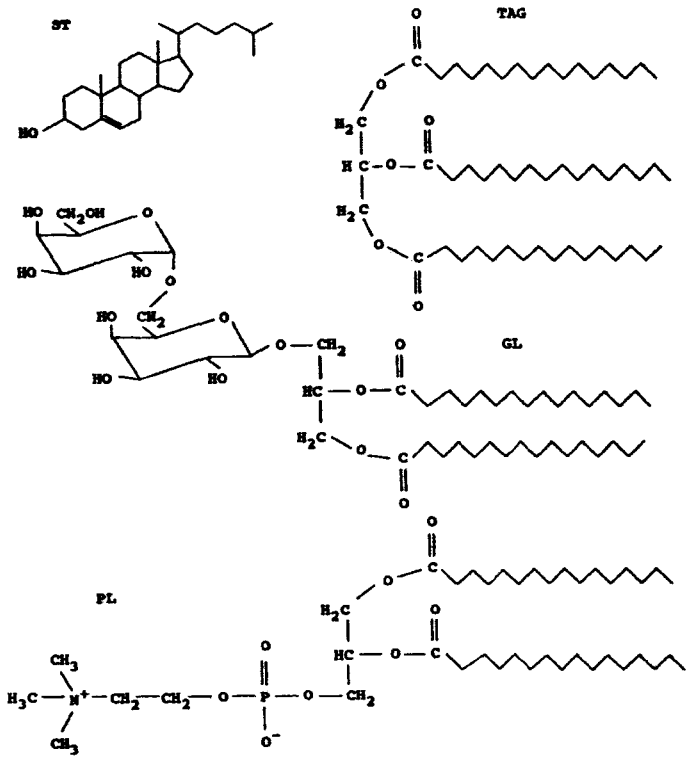


Figure 10.16 Structures of four lipid classes. PL=phospholipid, GL=glycolipid, TAG=triacylglycerol, ST=sterol. From Parrish (1999).

Fatty acids consist of a chain of carbon atoms with one methylated end (Figure 10.17 to Figure 10.19). There is a shorthand nomenclature for fatty acids: for example docosahexaenoic acid is 22:6ω3, where 22 is the number of carbon atoms in the chain, 6 is the number of double bonds, and 3 is the position of the first double bond (indicated by the omega symbol (ω)) starting from the methyl end (Napolitano 1999; Parrish 1999). A structural diagram of docosahexaenoic acid is given in Figure 10.19.

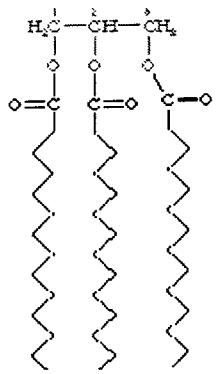


Figure 10.17 Structural diagram of a triacylglyceride (TAG); glycerol backbone in blue and fatty acid chains in black (From http://distance.stcc.edu/AandP/AP/AP1pages/Units1to4/epitissmol/lipids.htm).

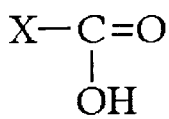


Figure 10.18 Methyl end of a fatty acid; X is where the carbon chain would extend; the glycerol backbone would join at the hydroxyl (-OH) group (From http://distance.stcc.edu/AandP/AP/AP1pages/Units1to4/epitissmol/lipids.htm).

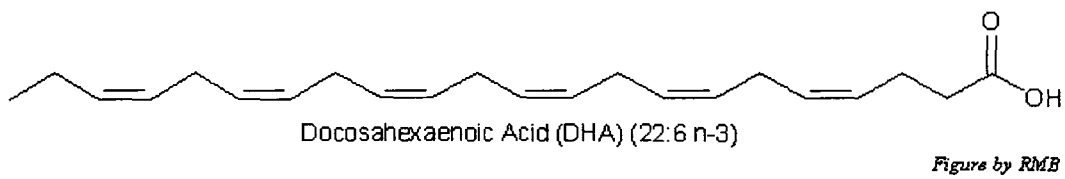


Figure 10.19 Structure of a long chain polyunsaturated fatty acid, docosahexaenoic acid (22:6ω3) (From lansbury.bwh.harvard.edu/ literature.htm).

Some fatty acids are essential to the normal functioning of cells, and these, or their precursors, must be obtained from an organism's diet. Animals are unable to place a double bond in the ω3 of the ω6 position (commonly referred to as omega3 and omega6) however they can elongate or desaturate fatty acids (Parrish 1999). Therefore ω3 and ω6

fatty acids must be obtained from food sources. Essential fatty acids (EFA) that cannot be synthesized by animals are 18:2ω6 and 18:3ω3, obtained from plant material (Parrish 1999). From these, longer essential fatty acids can be synthesized (Arts et al. 2001). Typical pathways for elongating these two essential fatty acids are: 1) 18:2ω6 to 20:4ω6 and 2) 18:3ω3 to 20:5ω3 and/or 20:4ω3 to 22:5ω3 to 22:6ω3 (Parrish 1999). Insects are capable of synthesizing these longer chain (with 20 carbon atoms or more) essential fatty acids (Bell et al. 1994; Stanley-Samuelson 1993). Adequate amounts of EFA are required for proper neural development in animals, and in humans for the prevention and mitigation of diseases such as cardiovascular and inflammatory diseases (Arts et al. 2001).

Fatty acids are also classified in terms of their degree of saturation. A completely saturated fatty acid (SAFA) has no double bonds (e.g. 16:0 or palmitic acid). Fatty acids with one double bond are termed monounsaturated (MUFA) (e.g. 18:1ω9 or oleic acid). Polyunsaturated fatty acids (PUFA) contain more than one double bond (e.g. 18:2ω6 or linoleic acid). Fatty acids with a high degree of unsaturation are termed HUFA (e.g. 20:5ω3, 22:5ω3, 22:6ω3) (Sushchik et al. 2003).

10.4 Appendix 4: Review of Hydropsychidae feeding ecology

Ingestion of detritus, plant and animal material by species of Newfoundland hydropsychids will be discussed to provide background for interpreting the lipid data and what it may indicate about differential uptake of potential food resources in a stream. A literature review of food uptake for each species was given in Chapter 1 with aspects of feeding discussed briefly throughout subsequent chapters. Hydropsychids are generally omnivores and feed on particulate organic matter (POM) (Wiggins 1996) which comprises phytoplankton, zooplankton, periphyton and bacteria (Hynes 1970b). Feeding experiments and gut content analysis from previous research showed food preferences amongst the species of Hydropsychidae. *Arctopsyche ladogensis* is the most carnivorous of the Newfoundland species (Wiggins 1996). *Hydropsyche betteni* is more carnivorous than other species of *Hydropsyche* (Fuller & MacKay 1980a). *Cheumatopsyche pettiti* has the finest mesh of the Newfoundland Hydropsychidae and so feeds on the finest material (Wallace 1975b). Other species of *Cheumatopsyche* feed primarily on plant and detrital matter with lesser amounts of animal material (Benke & Wallace 1980; Fuller et al. 1983; Fuller et al. 1988; Fuller & Mackay 1981; Petersen 1985; Rhame & Stewart 1976).

Amounts of carnivory may generally separate the diets of hydropsychids, as *Arctopsyche* is reportedly highly carnivorous (Wiggins 1996). Ross & Wallace (1983) quantified foregut contents of the following species in a stream in North Carolina (approximate percentages of fine detritus and animal material consumed in brackets): *P. apicalis* (64, 35), *D. modesta* (73, 16), *H. slossonae* (60, 32), *H. sparna* (51, 42) and *H. betteni* (58, 39). Proportions of animal material were similar for four of the five species,

and so competition among co-existing species may not be reduced via partitioning of food sources. However, Benke & Wallace (1980) found greater carnivory differences amongst the genera in a Georgian river (with percentage of carnivourous material in the gut in brackets): *A. irrorata* (73.1%), *P. cardis* (62.3%), *H. sparna* (23.5%) and *D. modesta* (19.3%), suggesting co-habiting species may partition food sources. It was suggested that *H. betteni* was more carnivorous than both *H. slossonae* and *H. sparna* in a southern Ontario stream because *H. betteni* nets had a greater total surface area (Fuller & MacKay 1980a), so physical differences may relate to food intake. *Hydropsyche sparna* and *D. modesta* were equally carnivorous in a Georgian stream, but *H. sparna* had less vascular plant material and more detritus than *D. modesta* (Wallace et al. 1977), thus there may be partitioning of non-animal food sources. Dietary intake is also influenced by space and time because (Haefner & Wallace 1981) reported that *D. modesta* fed mainly on vascular plant material and fine detritus. Thus it is not known if dietary differences are an artifact of the available food sources or if hydropsychids are selectively ingesting foods. Proportions of carnivorous gut contents did not greatly differ among co-occurring *A. irrorata* and *P. cardis* (Wallace et al. 1977), or *C. etrona* (43%) and *H. venularis* (31%), although *C. etrona* had more detritus and *H. venularis* more vascular plant material (Wallace 1975a). This is not true for all *Cheumatopsyche* species as *C. pettiti* consumed mostly detritus (>50%) with diatoms and other algae in a Hawaiian stream (Kondratieff et al. 1997) and detritus (~87%), animal material (~8%) and (~5%) algae in a Virginia stream (Sanchez & Hendricks 1997).

Hydropsychids also show seasonal dietary shifts. Mecom (1972) found *A. grandis* was highly carnivorous from June to August, and more of a diatom feeder during the Colorado winter. *Hydropsyche* sp. also showed a seasonal shift, feeding on diatoms in the Colorado winter, but detritus in the spring and summer. Fuller & MacKay (1980a) found *Hydropsyche* to ingest more animal material in the spring and summer, and that later instars consumed increased quantities of animal material as opposed to detritus (Fuller & MacKay 1980a). Ross & Wallace (Ross & Wallace 1983) also found later instars to have higher proportions of animal material. This change in diet where food preferences differ from early to later instars is known as an ontogenetic shift (Lancaster et al. 2005). This shift may be a product of the increase in net and mesh size with larger instars because this partly determines the size of food captured (Wallace & Merritt 1980).

Fuller & Mackay (1981) found that food quality affected larval growth. Animal material caused greater weight increases than diatoms or detritus. The importance of animal material to growth varied with season, instar and species. In general, earlier instars depended more on detritus. In the laboratory, *H. sparna* was able to utilize all food sources, and so grew at a faster rate than either *H. betteni* or *H. slossonae*. This may account for its widespread distribution. Petersen (1985) found three species selected *Daphnia* over detritus, where the larvae would reject detritus particles but continue to eat *Daphnia*. Fuller et al. (1988) also reported selective feeding, where *Ulothrix* was ingested over *Chlorella* with the former contributing to higher weight gain and thus may have had a higher nutritional value.

10.5 Appendix 5: Fatty acid markers in freshwater ecosystems

An ideal marker would be distinctive in terms of its origin, easily identifiable, not processed during ingestion and tissue incorporation, and remain metabolically stable as it is transferred up the food chain. Fatty acid markers are not this ideal because they are not always easily identifiable nor directly incorporated and stable throughout the food web. However, they do allow investigation of food intake over a longer period of time (Dalsgaard et al. 2003). Most research on fatty acid markers has been conducted in marine environments. Application of these markers in freshwater systems should be approached with caution since phytoplankton and zooplankton groups in marine waters may have a very different composition than those in freshwater. Leveille et al. (1997) developed specific markers for lake phytoplankton in France by expanding upon marine fatty acid markers for diatoms, dinoflagellates and green algae. Research on lentic phytoplankton is applicable to the present study because a primary focus here is on ingestion of seston which may originate from lakes. Napolitano (1999) gives a comprehensive overview of fatty acid research on freshwater algae, phytoplankton, zooplankton and bacteria. A single fatty acid is generally not indicative of a group of organisms, rather a fatty acid profile is used to differentiate classes (Napolitano 1999). This profile may comprise several fatty acids in ratios which create a distinctive marker. These markers have been developed in laboratory experiments and in natural populations where the fatty acid composition of the diet of an organism is analyzed and compared to the organism's fatty acid composition to determine similarities. Any such similarities would indicate a transfer of fatty acids between the trophic levels (Cripps & Atkinson

2000; Dwyer et al. 2003; Henderson et al. 1996; Stevens et al. 2004b). This relationship is generally easier to discern at lower trophic levels (Dalsgaard et al. 2003), often from phytoplankton to carnivorous zooplankton although extensive work has also been conducted with fish and species used for aquaculture.

Fatty acid compositions of organisms vary with time of year as available food resources change. For example, spring diatom blooms are reflected in the fatty acid composition of their predators because of greater uptake of diatoms in the spring compared to the summer (Leveille et al. 1997). Fatty acid compositions of organisms are greatly affected by temperature, light and nutrients (Napolitano 1999). Low temperatures increase the level of unsaturation to maintain membrane fluidity (Brett & Muller-Navarra 1997). Stress caused by non optimal light levels increases production of saturated and ω6 fatty acids and decreases production of ω3 fatty acids, which reduces the quality of the phytoplankton as a food source to higher trophic levels (Ahlgren et al. 1992). Nitrogen and phosphorous are essential for phytoplankton growth, so low levels of these interfere with biochemical pathways and fatty acid production and storage, which alters the overall fatty acid composition of organisms (Ahlgren et al. 1997). Because of all these variables, development of fatty acid markers specific to a given set of conditions in an area is not practical. Despite this, several fatty acid markers are relatively robust. Groups of organisms often have more than one fatty acid marker and studies often use multiple markers to verify the presence/dominance of a group (Dalsgaard et al. 2003).

Plants produce glycolipids which have a fatty acid composition rich in ω3 PUFA. Heterotrophs have limited abilities to synthesize these fatty acids which are essential to

growth and development. Therefore they must be acquired in the diet. Plants are generally the only organisms which can synthesize 18:2ω6 (linoleic acid. LA) and 18:3ω3 (α-linolenic acid, ALA), derived from C_{16} PUFAs, and so these fatty acids can be used as markers in higher trophic levels. They are precursors to essential PUFAs via the following pathways: 18:2ω6 to 18:3ω6 to 20:3ω6 to 20:4ω6 (arachidonic acid, ARA) to 22:4ω6 to 22:5ω6; and 18:3ω3 to 18:4ω3 to 20:4ω3 to 20:5ω3 (eicosapentenoic acid, EPA) to 22:5ω3 to 22:6ω3 (docosahexaenoic acid, DHA). Organisms differ in their ability to elongate C_{18} to C_{20+} PUFAs in amounts required for growth and reproduction. Thus this group of fatty acids is termed essential fatty acids (EFAs).

In marine environments phytoplankton communities are predominantly diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae) and coccoliths (Prymnesiophyceae); while macroalgae communities are mainly green algae (Chlorophyceae), red algae (Rhodophyceae) and brown algae (Phaeophyceae) and so fatty acid markers of these organisms have been developed. These organisms match some of the major classes of freshwater algae/phytoplankton including diatoms, dinoflagellates, green algae, cyanobacteria (Cyanophyceae) and golden brown algae (Chrysophyceae) (Napolitano 1999). From this assortment, material ingested by hydropsychids likely consists of diatoms, dinoflagellates, green algae and golden brown algae and so fatty acid markers for these species are of interest here.

General indicators of algae are PUFAs, particularly the ω3 PUFAs (18:4ω3, 18:5w3, 20:5ω3, 22:6ω3) because they are absent from both bacteria and terrestrial plants, are retained by animals and their synthesis in animals is limited. There are several

markers for diatoms derived from marine samples. Fatty acids with a carbon chain length of 16 (C_{16}) and ω3 PUFAs, particularly 20:5ω3 are generally indicative of diatoms (Parrish et al. 2000). Ratios of 16:1/16:0 > 1.6, $\Sigma C_{16}/\Sigma C_{18}$, and the fatty acid 16:4ω1, a fatty acid commonly found in diatoms but not other phytoplankton, were proposed by Claustre et al. (1989) as diatom markers. Other proposed diatom markers are 16:1ω7/16:0 >1 (Jeffries 1970). However, 16:1ω7 is elevated in senescent cells and so diatom physiology may limit the applicability of this marker. Leveille et al. (1997) suggested a sum of C_{16} PUFAs for freshwater diatoms (16:2ω4 + 16:3ω4 + 16:4ω3 + 16:4ω1). They also found a higher proportion of 14:0 and suggested a ratio of (14:0 + 16:1ω7 + 16:2ω4 + 16:3ω4 + 16:4ω1)/16:0. Diatoms are ubiquitous in freshwater, occupying both planktonic and benthic habitats and exhibit blooms in the late spring/early summer in temperate regions (Scruton et al. 1987; Sheath & Wehr 2003).

Dinoflagellates generally have elevated levels of 22:6ω3 and the ratio of 22:6ω3/20:5ω3 shows the prevalence of dinoflagellates in relation to diatoms (Budge & Parrish 1998). They generally have higher levels of C_{18} especially 18:0, 18:1ω9, 18:4ω3 and 18:5ω3 so the sum of C_{18} and C_{22} ω3 PUFAs (18:4ω3+18:5ω3+22:5ω3+22:6ω3) is also indicative of dinoflagellates (Dalsgaard et al. 2003; Parrish et al. 2000). Dinoflagellates are able to shorten 20:5ω3 to 18:5ω3, indicating the importance of these two fatty acids as markers of this group. The presence of these fatty acids may depend on the physiology of the cells and thus Leveille et al. (1997) suggested a ratio of (16:0 + 18:4ω3 + 20:5ω3 + 22:6ω3) / (18:3ω3 + 16:2ω4 + 16:3ω4 + 16:4ω3 + 16:4ω1). Because green algae have elevated amounts of 18:3ω3 compared to dinoflagellates, higher values

of the ratio 18:5ω3/18:3ω3 indicate a prevalence of dinoflagellates (Dalsgaard et al. 2003). Dinoflagellates are planktonic in lentic bodies and bloom when nitrogen and phosphorous levels are high (Sheath & Wehr 2003). They occur in low proportions in Newfoundland headwater lakes (Scruton et al. 1987).

The fatty acid composition of green algae is more similar to higher plants than to other eukaryotic algae and so higher levels of 18:3ω3 in combination with higher levels of 18:2ω3 and a ratio of 18:1ω7/18:1ω9 >1 are indicative of this class (Dalsgaard et al. 2003; Parrish et al. 2000). Leveille et al. (1997) found that the lacustrine green alga *Oocytis lacustris* (found in Newfoundland lakes) had higher concentrations of 16:3ω3, 18:3ω3 and 20:4ω3 compared to other algal groups. Ahlgren et al. (1992) found 16:0 and 18:3ω3 in four species of green algae, generalizing ω3 fatty acids to have higher levels than ω6 fatty acids and used the ratio of ω3/ω6 >=2 as indicative of this group. Green algae are part of the planktonic and benthic community, occurring widely in inland waters (Sheath & Wehr 2003). A number of taxa occur in Newfoundland headwater lakes (Scruton et al. 1987).

Golden brown algae had a very high ratio of ω3/ω6 (>18) and unusually high amounts of 16:0 and 18:1ω9 (Ahlgren et al. 1992). However, these two fatty acids are also found in other organisms and are not solely attributable to this group. Golden brown algae can comprise almost half the phytoplankton biomass in Newfoundland headwater lakes, which provide ideal habitats for them because of their low to moderate nutrient levels, low conductivity and slightly acidic pH levels (Scruton et al. 1987; Sheath & Wehr 2003).

Marine cyanobacteria are characterized by ~20% 18:1ω7, but freshwater species had a much lower level of this fatty acid (0.5-3.1%) (Ahlgren et al. 1992; Napolitano 1999). A second characteristic fatty acid of freshwater cyanobacteria is 18:3ω6 (Napolitano 1999). Cyanobacteria form surface blooms in nutrient-rich waters thus dominating the algal community. The chemical and physical composition of Cyanobacteria often renders this group toxic and unpalatable to zooplankton and fish and so it will not be further explored (Sheath & Wehr 2003).

Bacteria are generally indicated by odd-numbered, hydroxyl and cyclopropane branched-chain fatty acids including 15:0*i*, 15:0*ai*, 15:0, 15:1, 16:0*i*, 16:0*ai*, 17:0*i*, 17:0*ai* and 17:1, with the last also a specific indicator of sulphate reducing bacteria (Napolitano 1999). Eukaryotic bacteria can have elevated levels of 16:1ω7 and 18:1ω7, with 18:1ω9 also present (Dalsgaard et al. 2003; Napolitano 1999).

Terrestrial plants have higher levels of 18:2ω6 and 18:3ω3. Low levels of these fatty acids were in river seston samples in the early summer with levels increasing in the late summer and fall when terrestrial plants undergo abscission (Parrish et al. 2000). If the sum of these two fatty acids is greater than 2.5, levels seen in the late summer and fall, then this is indicative of terrestrial material (Budge & Parrish 1998). Another suggested marker is the sum of 22:0 and 24:0 (Budge et al. 2001). The sum of very long chain saturated fatty acids ($\Sigma C_{24:0} - \Sigma C_{32:0}$) is indicative of terrestrial plants (Meziane et al. 1997). Thus the ratio of very long chain fatty acids to medium chain fatty acids (~C_{16}), found in phytoplankton, is indicative of the proportion of allochthonous to autochthonous material (Napolitano 1999).

Deriving fatty acid markers unique to carnivory at higher levels in the food chain is difficult because markers tend to become less clear after metabolic processing and selective incorporation up the food chain (Dalsgaard et al. 2003). In freshwater systems phytoplankton is the diet of herbivorous and omnivorous zooplankton. Phytoplankton is assimilated mostly unaltered by herbivorous zooplankton. Zooplankton tend to store fats as triacylglycerols (~20%) and sterol and wax esters (~80%) (Cavaletto et al. 1989). Sterols and wax esters consist of 20:1 and 22:1 monounsaturated fatty acids and thus the sum of these are a fatty acid marker for herbivorous zooplankton (Dalsgaard et al. 2003). Omnivorous and carnivorous zooplankton synthesize 16:0 and 18:0, and the latter is desaturated to 18:1ω9, a fatty acid used as a general carnivory marker in marine systems. However, it is also present in freshwater bacteria, dinoflagellates and green algae and so is not a unique indicator of carnivory. In marine systems the ratio of 18:1ω7/18:1ω9 <1 is used as a carnivory marker, although some algae have higher levels of 18:1ω9 and this fatty acid is utilized during periods of starvation, so this marker should be used with caution (Dalsgaard et al. 2003). A modification of this is the ratio of 18:1ω9/(18:1ω7 + 16:1ω7) >1 because 18:1ω7 can be elongated from 16:1ω7 which is prevalent in diatoms and so the denominator may be more representative of a herbivorous diet (Falk-Petersen et al. 2000). The fatty acid 22:6ω3 (DHA) is conserved throughout the food chain, whereas 20:5ω3 is not, and so the ratio of 20:5ω3/22:6ω3 (EPA/DHA) will decrease at higher trophic levels (Dalsgaard et al. 2003). However, hydropsychids are fairly low on the food chain and would have higher levels of 20:5ω3 if consuming diatoms, assuming this is an appropriate fatty acid marker for that algal class. A similar carnivory marker is

the ratio PUFA/SAFA >1 because PUFAs are conserved at higher levels in the food chain (Dalsgaard et al. 2003). This ratio was first suggested by Cripps & Atkinson (2000) because it was found to increase significantly when Antarctic krill were fed a carnivorous diet for 16 days. No definitive fatty acid markers for carnivory in freshwater have been found. The applicability of these marine markers to freshwaters is speculative because carnivorous freshwater fish are able to synthesize small amounts of PUFAs whereas marine species cannot and so these compounds are not conserved in freshwater food webs as they are in their marine counterparts (Kainz et al. 2004).

A study of pond amphipods found them to be a rich source of EPA and DHA (Arts et al. 2001) and are thus a potential source of these compounds for hydropsychids. Investigation of freshwater fatty acid markers beyond the level of consumption by zooplankton is limited, with some research conducted on freshwater fish. There are only a few reports on lipids in stream and river habitats (Bell et al. 1994; Hanson et al. 1985; Sushchik et al. 2003). Fatty acids of freshwater bacteria, algae and phytoplankton have been studied as well as their consumption by zooplankton. However, little work has been done at the trophic level of benthic invertebrates. No fatty acid markers have been tested in Newfoundland freshwaters. Note that most of Newfoundland freshwater is low in nutrients and the climate is cool, factors which affect fatty acid composition and thus the fatty acid markers chosen may not adequately represent the food supply of Hydropsychidae.